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Modeling the kinetics of peroxidase inactivation, colour and texture changes of Portuguese cabbage (*Brassica oleracea* L. var. *costata* DC) during UV-C light and heat blanching

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Abstract

The effects of heat blanching and UV-C light followed by heat on Portuguese cabbage peroxidase (POD), colour and texture were studied in the temperature range of 80–95 °C. POD inactivation, lightness (*L*) and yellowness (*b*) colour changes were described by a first-order reaction model. The greenness (*a*) colour and texture (firmness) changes followed a two fraction kinetic model behaviour. The temperature effect was well described by the Arrhenius law.

At lower temperatures the combined treatment showed higher POD inactivation. Colour and texture parameters did not show significant differences between treatments. Long processing times turned the leaves slightly darker, decreased greenness, yellowness and firmness. Short processing times increased the firmness and greenness of the leaves. The treatment at 80 °C for 90 seconds reduced 90% of POD, retaining 98% of lightness and 92% of yellowness and improved the green colour (130%) and firmness (125%). At 80 °C the heat blanching required 7.4 min to inactivate 90% of the enzyme activity, reducing lightness, greenness, yellowness and firmness to 92%, 68%, 62% and 61%, respectively. The present findings will help to optimize the Portuguese cabbage blanching conditions.

Keywords: Portuguese cabbage; Blanching; UV-C light; Peroxidase; Colour; Texture

1 Introduction

Portuguese cabbage (*Brassica oleracea* L. var. *costata* DC) is a very popular cabbage in Portugal together with galega cabbage, all very rich in vitamins and mineral salts, being the major ingredient in the traditional kale soup and in the “cozido à portuguesa” (traditional dish consist-

ing of boiled cabbage with sausages, beef and pork). This cabbage is a perishable product and the application of a freezing process could be a good solution to extend its shelf-life. Many fruits and vegetables are blanched (e.g. immersion in hot water for a short time) before any freezing process, to inactivate oxidative enzymes. Peroxidase (POD) is one of them and acts on the hy-

drogen peroxide (H_2O_2) present in the cell, leading to oxidation of several cellular compounds, thus causing changes of colour and flavour in vegetables (Nagodawithana & Reed, 1993). Due to its resistance to thermal treatment, POD is often used as a blanching indicator (Ganjloo, Rahman, Osman, Bakar, & Bimakr, 2011; Szymanek, 2011).

The blanching process has proved to be one of the most effective processes to clean the surface of vegetables, destroy microorganisms and inactivate enzymes. On the other hand, blanching may also cause off-flavours, off-colours, changes in texture, vitamins and minerals (Patras, Tiwari, & Brunton, 2011). Moreover, from an industrial point of view, this process may be extremely water and energy consuming (Aguilar, Rodriguez-Herrera, Montanez-Saenz, Reyes-Vega, & Contreras-Esquivel, 2004). Non-thermal treatments, aimed at reducing microbial content or enzyme activity, while retaining the quality of the product, are promising alternative technologies in food processing. An emerging and challenging technology that can be used by the food industry is the application of ultraviolet (UV) radiation (Allende & Artes, 2003). Low intensity light treatments may modify the conformation of the enzyme active site without changing its catalyzing capacity but leading to an increase of the overall activity. On the other hand, enzyme inactivation may be the result of non-reversible structural changes due to higher intensity light treatments (Manzocco, Dri, & Quarta, 2009). Perhaps the weakest point of UV-C light is its poor penetrative capacity, therefore this technique is limited to food surface applications. Nevertheless, the application of physical factors, such as temperature and/or pressure, combined with UV-C light, may result in the increase of the enzymatic activity, followed by further inactivation. These techniques used in combination (or sequentially), normally referred to as hurdle technologies, have been used as less severe heat treatments, resulting in higher quality and safer products than traditional treatments (Manzocco, Dri, & Quarta, 2009). Moreover, UV-C light has a positive image to the consumer and is considered a low cost non-thermal technology (Koutchma, 2009).

On the basis of these considerations and the few

studies available in the literature reporting the effect of blanching treatments on cabbage leaves (Alvarez-Jubete, Valverde, Patras, Mullen, & Marcos, 2014; Jaiswal, Gupta, & Abu-Ghannam, 2012), the aim of this work was to evaluate the kinetics of Portuguese cabbage POD inactivation, colour and texture changes during heat blanching and an alternative blanching process (UV-C light followed by heat blanching) and with the findings help to optimize the Portuguese cabbage blanching conditions.

2 Materials and Methods

2.1 Sample preparation

Portuguese cabbage was purchased at a local market in Faro, Portugal. The leaves were selected and the tip of each leaf was cut and then weighed (11 g).

2.2 Heat blanching and combined treatment processes

The samples were separated in two groups according to the treatment to be applied: UV-C light followed by heat blanching group and the heat blanching group. The first group was exposed to a UV-C radiation intensity of 11 Wm^{-2} for 90 s (each leaf side) at $25 \pm 1 \text{ }^\circ\text{C}$ in a ventilated UV-C chamber (composed of four UV lamps (Philips TUV G8T5 8 W, Holland), each separated by 10 cm). The lamps were placed at 21.5 cm above the processing surface. During UV-C radiation processing, lab coats, protective gloves and safety goggles were worn by staff. UV-C radiation intensity was determined by a photo-radiometer (Delta OHM DO 9721, Caselle di Selvazzano, Italy) and an irradiance probe (Delta OHM LP 9021 UVC, Caselle di Selvazzano, Italy). Then, both groups were immersed in an 8 L water bath (Grant W14, Cambridgeshire, England), with temperatures ranging from 80 to 95 $^\circ\text{C}$ for different periods of exposure (up to 480 s, based in preliminary tests for peroxidase inactivation). After the thermal treatments, the leaves were cooled down in an iced water bath. The temperature was monitored by a digital thermometer (Ellab ctd 87,

Roedovre, Denmark) and a thermocouple (1.2 mm needle dia.; constantan-type T). The experiments were run in triplicate.

2.3 Peroxidase determination

Peroxidase extraction

Leaves (3 g) were added to 100 ml of potassium phosphate buffer solution, previously prepared with distilled water and mono and dipotassium phosphate (Merck) with a molar concentration of 0.1 M, pH 6.5 and kept at 4 °C (according to the method of (Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002), and homogenized for 1 minute with an Ultra-Turrax (IKA T25 Janke & Kunkel, Staufen, Germany). The suspension was then centrifuged in polypropylene tubes for 10 minutes at 18,000×g (Sigma 3 K20, Osterode, Germany) and 4 °C. The POD enzymatic extract was then filtered through filter paper Whatman n^o 1.

Peroxidase activity measurement

The POD activity was determined by mixing 0.120 ml of enzymatic extract and 3.48 ml of substrate solution in 10 mm-path-length glass cuvettes (Amersham Bioscience). The substrate solution was prepared daily (mixture of 0.1 ml of guaiacol (BDH Chemicals Ltd) (99.5%), 0.1 ml of hydrogen peroxide (Panreac) (30%) and 99.8 ml of potassium phosphate buffer (0.1 M, pH 6.5)). The POD activity was measured by absorbance increase using an UV/Vis spectrophotometer (Hitachi U-2000, Tokyo, Japan) at 6 s intervals and 470 nm (absortivity $\xi=26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ for tetraguaiacol). The reaction was followed until steady state was reached using a blank prepared with 0.120 ml of distilled water and 3.48 ml of substrate solution.

Protein determination

The protein content was determined by Lowry's method using bovine serum albumin (BSA) (Fluka) as standard. The results were expressed as mg of total protein and the enzyme specific activity was expressed as $\mu\text{mol min}^{-1} \text{ mg protein}^{-1}$.

Colour determination

Colour was evaluated with the Hunter Lab coordinates [L (lightness, 0 for black to 100 for white), a (red-green) and b (yellow-blue)] with a tristimulus colorimeter (Dr Lange Spectrocolour, Berlin, Germany) (Hunterlab, 2012). The colorimeter (d/8° geometry, illuminant D65, 10° observer) was calibrated against a standard ceramic white tile (X=84.60, Y=89.46, Z=93.85) and a standard ceramic black tile (X=4.12, Y=4.38, Z=4.71). Measurements were taken in triplicate with 5 readings each.

Texture determination

The texture of raw and processed samples was analysed using a texturometer (LFRA Texture Analyser, Brookfield) equipped with a 1.5 kg load cell. Based on the method reported by Tanigaki, Takahashi, Sakurai, Takada, and Nagata (2009), each leaf was sandwiched between two metal plates with a cylindrical hole in the centre (diameter=15 mm). The test speed was set at 2 mm/s and a stainless steel probe (TA-39) with flat end, 2 mm diameter and 20 mm long was used to penetrate the sample. Peak force was used to quantify the firmness (g) of the samples. Measurements were run in triplicate.

pH determination

Two grams of raw Portuguese cabbage were homogenized (Ultra-Turrax T25 Janke and Kunkel) for 1 min at 13,500 rpm in 20 ml of degassed water (Health Canada-Official Method, 1999). The extracts were obtained through agitation with a mechanical shaker (Edmund Bühler 7400 Tübingen KL2) for 30 min. Afterwards, samples were kept in the dark for an additional hour. The supernatant was decanted and the pH was measured (pH meter Crison micropH 2002) in ten replicates.

Data analysis

Enzyme inactivation, colour and texture changes are well described in the literature by zero, first-order, fractional and two fraction first-order models (Anthon & Barrett, 2002; Cruz, Vieira, & Silva, 2006; Gonçalves, Pinheiro, Abreu,

Brandão, & Silva, 2007; Lau, Tang, & Swanson, 2000; Morales-Blancas et al., 2002; Soysal & Soyilemez, 2005; Tijssens, Schijvens, & Biekman, 2001). After preliminary analysis, a first-order reaction model (Eq. 1) was used to fit the experimental data for POD inactivation and L and b parameters colour changes obtained for each blanching process.

$$C_t = C_0 e^{-kt} \quad (1)$$

Where C_0 is the initial value and C_t the value at time t .

The temperature dependence of the rate constant (k) followed the Arrhenius behaviour (Eq. 2).

$$k = k_{ref} e^{\left[-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} \quad (2)$$

A global model (Eq. 3) was obtained replacing k in Eq. 1 by the Arrhenius equation (Eq. 2) (Arabshahi & Lund, 1985):

$$\frac{C_t}{C_0} = e^{\left[-k_{ref} e^{\left[-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} t \right]} \quad (3)$$

For texture and a parameter colour changes, a two fraction first-order reaction model (Eq. 4) was used to fit the experimental data.

$$C_t = C_{01} e^{-k_1 t} + C_{02} e^{-k_2 t} \quad (4)$$

For both fractions, it was assumed that the first-order rate constants, k_1 and k_2 , dependence on temperature (Eqs. 5 and 6) followed the Arrhenius law:

$$k_1 = k_{ref1} e^{\left[-\frac{E_{a1}}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} \quad (5)$$

$$k_2 = k_{ref2} e^{\left[-\frac{E_{a2}}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} \quad (6)$$

A global model (Eq. 7) was obtained replacing k in Eq. 4 by the Arrhenius equation (Eq. 5 and

6) for both fractions.

$$C = C_{01} e^{\left[-k_{ref1} e^{\left[-\frac{E_{a1}}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} t \right]} + C_{02} e^{\left[-k_{ref2} e^{\left[-\frac{E_{a2}}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} t \right]} \quad (7)$$

Experimental data points were normalized, by dividing the values at time t (C_t) by the initial value (C_0). An analysis of variance (two-way ANOVA with replication) was performed to assess the effect of blanching time-temperature (tested factors-independent variables) conditions on POD activity, colour and texture changes (dependent variables). The kinetic parameters were estimated directly from the experimental data, by performing a one-step non-linear regression analysis (using least squares estimation and Levenberg–Marquart method), fitting the global model equations 3 (first-order reaction) and 7 (two fractions first-order reaction) (Arabshahi & Lund, 1985) and using the statistical software STATA version 10.0. The reference temperature (T_{ref}) was considered to be the average temperature of the experiments (87.5 °C) aimed at improving parameter estimation. The goodness of fit of the regression was assessed by the coefficient of determination (R^2) while normality and randomness of residuals by the predicted vs. experimental data chart; the precision of the parameters was evaluated by confidence intervals at 95%.

3 Results and Discussion

3.1 Peroxidase inactivation

Raw Portuguese cabbage presented a peroxidase activity of $0.30 \pm 0.17 \mu\text{mol min}^{-1} \text{mg protein}^{-1}$ and a pH of 6.32 ± 0.12 . Experimental and predicted values of the POD activity for heat only and the combined treatment are presented in Figure 1. The results at higher temperatures, 90 and 95 °C, showed similar rate of POD inactivation between heat and the combined treatment (about 100% inactivation in the first 10 s

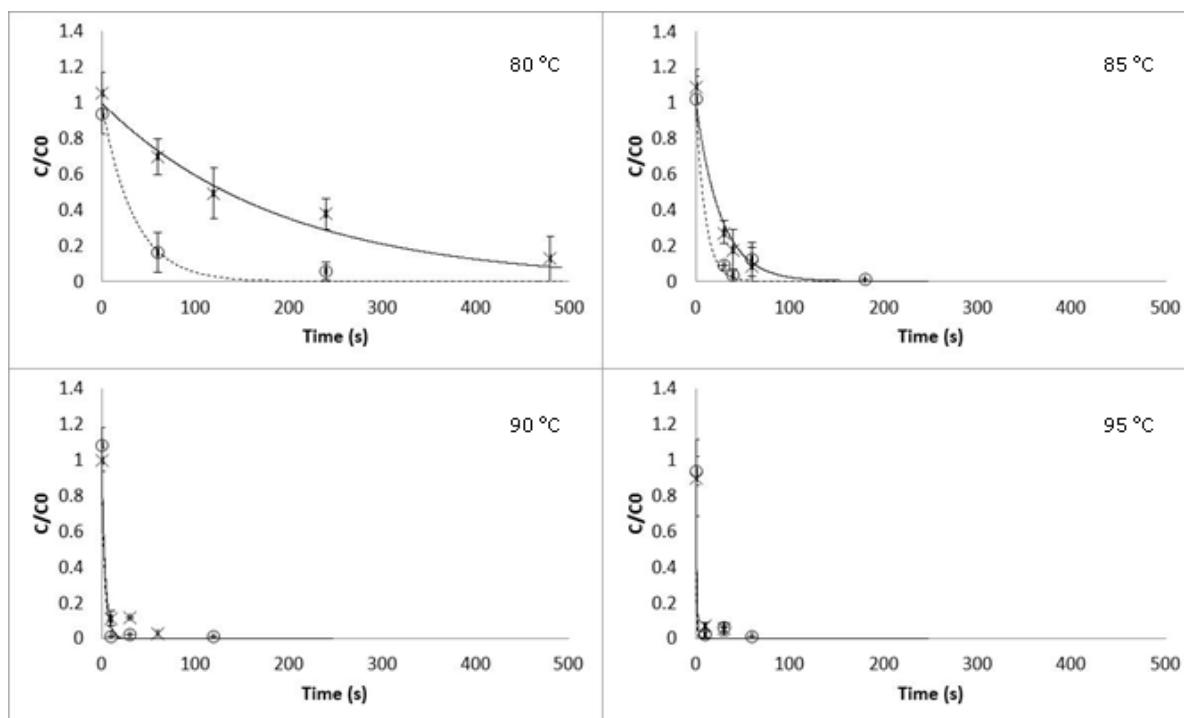


Figure 1: Effect of temperature and UV-C light over time on POD specific activity in Portuguese cabbage in the temperature range 80-95 °C: (x) experimental values of POD specific activity with heat blanching processes; (—) model predicted values for heat blanching processes; (o) experimental values of POD specific activity with combined UV-C light and heat blanching processes; (---) model predicted values with combined UV-C light and heat blanching processes. Bars represent mean \pm standard deviation (n=3)

of processing), meaning that the high temperature was the main factor for this outcome. Nevertheless, at lower temperatures (80 and 85 °C) the combined treatment showed a higher POD inactivation rate (about 4-fold at 80 °C and 1 min of exposure) due to the synergistic hurdle effect produced by the UV-C light followed by heat. These results were mainly due to the structural changes on the enzyme active site, leading to a lower enzyme-substrate interaction. Lamikanra, Kueneman, Ukuku, and Bett-Garber (2005) found that UV treatment increases POD activity while lowering esterase and lipase activity. In another study, Manzocco, Quarta, and Dri (2009) showed that exposure of apple derivatives to UV-C light is very effective in polyphenoloxidase inactivation. These outcomes are dependent

on the applied methodologies and the different types of food matrices.

Concerning the kinetic modelling, a first-order kinetic model was well-fitted to results obtained for both blanching processes ($R^2=0.97$ and $R^2=0.98$ in table 1), meaning that the enzyme inactivation could be described by this model. Other studies also proposed this model for POD inactivation, for watercress (Cruz et al., 2006), for carrots (Soysal & Soylemez, 2005) and for broccoli (Morales-Blancas et al., 2002). Also, the temperature dependence of the rate constant followed the Arrhenius behaviour. The thermal inactivation reaction rate (k_{ref}) and activation energy (Ea) for heat and UV-C+heat blanching treatments are shown on Table 1. The reaction rate for the combined treatment, at the refer-

Table 1: Kinetic parameters for quality changes in Portuguese cabbage

	Heat blanching	UV-C and heat blanching
<i>Peroxidase</i>		
k_{ref} (s^{-1})	0.10±0.02	
	0.18±0.06	
Ea ($kJ mol^{-1}$)	417.80±34.88	255.96±49.08
<i>Colour (L)</i>		
k_{ref} (s^{-1})	0.00041±0.00009	0.00040±0.00012
Ea ($kJ mol^{-1}$)	117.9±35.74	63.96±49.42
<i>Colour (a)</i>		
C_{01}	1.62±0.13	1.44±0.12
$155.33±16.75k_{ref1}$ (s^{-1})	0.0056±0.00095	0.0054±0.00040
Ea_1 ($kJ mol^{-1}$)	0.63±0.13	0.45±0.18
C_{02} 149.62±10.43		
k_{ref2} (s^{-1})	0.082±0.038	0.076±0.043
Ea_2 ($kJ mol^{-1}$)	79.98±52.41	68.95±41.24
<i>Colour (b)</i>		
k_{ref} (s^{-1})	0.0021±0.0005	0.0017±0.0003
Ea ($kJ mol^{-1}$)	96.60±35.60	
	144.95±31.11	
<i>Firmness (g)</i>		
C_{01}	0.55±0.12	0.58±0.10
k_{ref1} (s^{-1})	0.11±0.06	0.13±0.08
Ea_1 ($kJ mol^{-1}$)	186.86±53.91	140.10±67.09
C_{02}	1.55±0.11	1.61±0.10
k_{ref2} (s^{-1})	0.0055±0.00098	0.0067±0.00088
Ea_2 ($kJ mol^{-1}$)	135.68±12.72	138.99±9.51

ence temperature, was higher ($0.18±0.06 s^{-1}$) than the value obtained for the heat blanching treatment ($0.10±0.02 s^{-1}$). The estimated activation energy for POD inactivation was in accordance with the ones reported by Anthon and Barrett (2002) $480 kJ mol^{-1}$ and $478 kJ mol^{-1}$ for carrot and potato. In contrast, Soysal and Soylemez (2005) reported values of $148 kJ mol^{-1}$ for POD inactivation in carrot. In another study, Cruz et al. (2006) reported activation energy values of $Ea_1 = 421 kJ mol^{-1}$ and $Ea_2 = 352 kJ mol^{-1}$ in watercress leaves, for heat-labile and heat-resistant fractions, respectively. Moreover, Neves, Vieira, and Silva (2012) reported that UV-C light also contributed to

the increase in POD inactivation rate in zucchini. The estimated kinetic parameters were $k_{Heat} = 7.37×10^{-7} min^{-1}$ and $Ea_{Heat} = 925 kJ mol^{-1}$ and $k_{Heat+UV-C} = 2.42×10^{-5} min^{-1}$ and $Ea_{Heat+UV-C} = 596 kJ mol^{-1}$.

3.2 Colour changes

Raw Portuguese cabbage presented values of L , a and b of $36.89±2.73$, $-7.49±1.17$ and $8.39±2.31$, respectively. At the end of each blanching process, these colour parameters did not show significant differences between treatments (ANOVA, $p>0.05$). The L and b colour parameters de-

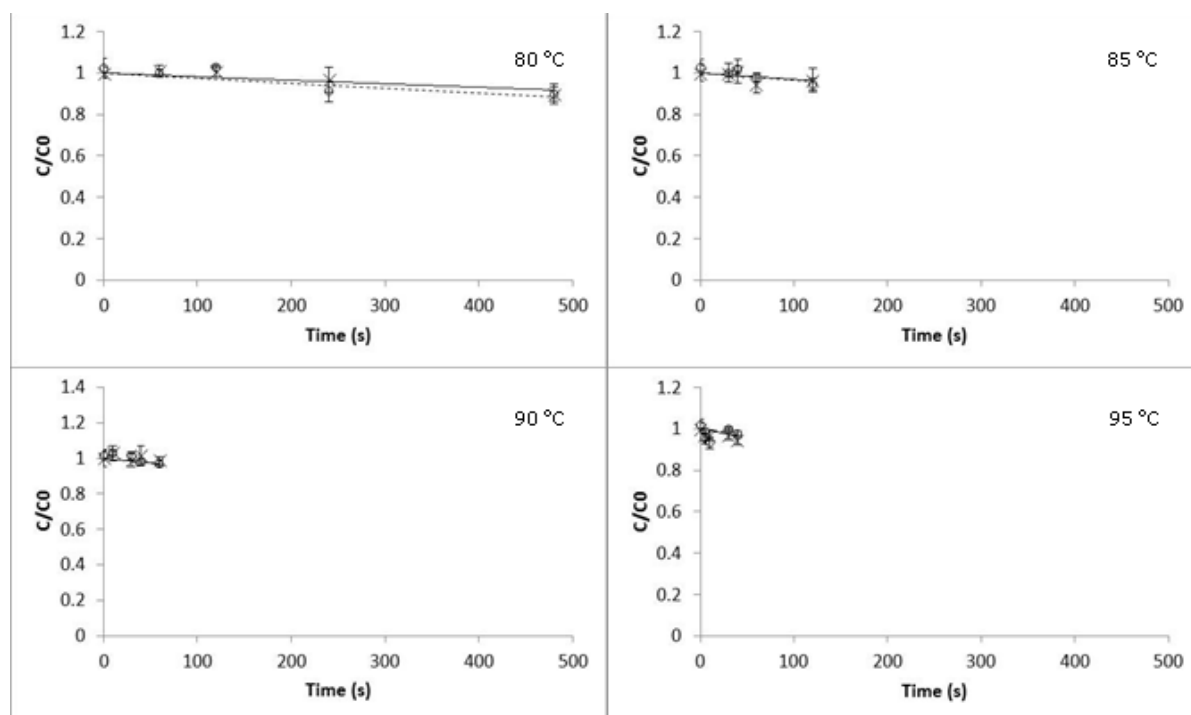


Figure 2: Effect of temperature and UV-C light over time on colour L parameter in Portuguese cabbage in the temperature range 80-95 °C: (x) experimental values of L parameter with heat blanching processes; (—) model predicted values for heat blanching processes; (o) experimental values of colour L parameter with combined UV-C light and heat blanching processes; (---) model predicted values for with combined UV-C light and heat blanching processes. Bars represent mean \pm standard deviation (n=15)

creased in both treatments (traditional and UV-C light followed by heat blanching), showing that the samples after blanching became slightly darker and less yellow (Fig. 2 and Fig. 4, respectively). Changes in the a colour parameter showed two distinct mechanisms. First, the a values increased right at the beginning of the blanching treatments (5-60 s and 80-95 °C) resulting in greener samples, and then decreased after some time of exposure turning the samples less green (Fig. 3). This decrease might be related to the chlorophylls leaching out into the blanching medium and/or conversion of chlorophylls to pheophytins (Schwartz & Vonelbe, 1983). These results are in agreement with those reported by Di Cesare, Forni, Viscardi, and Nani (2004), in which leaves of oregano also became greener after blanching. Lin

and Brewer (2005) showed in a study with frozen peas that after microwave and water blanching the peas were also greener. In another study, Brewer, Begum, and Bozeman (1995) reported significant darkening of stems and florets of broccoli after microwave blanching. Tijskens et al. (2001) presented similar effects on green colour of blanched broccoli and green beans. Recently, Alvarez-Jubete et al. (2014), in a study in which white cabbage samples were blanched at 90-95 °C during 3 min, also reported a slight decrease in the L parameter. These colour changes may be related to the replacement of the gases inside the intercellular spaces by the blanching medium, altering light refraction from the cell surface (MacDougall, 2002).

In terms of kinetic modelling, a first-order model and two fractions first-order model fitted well the

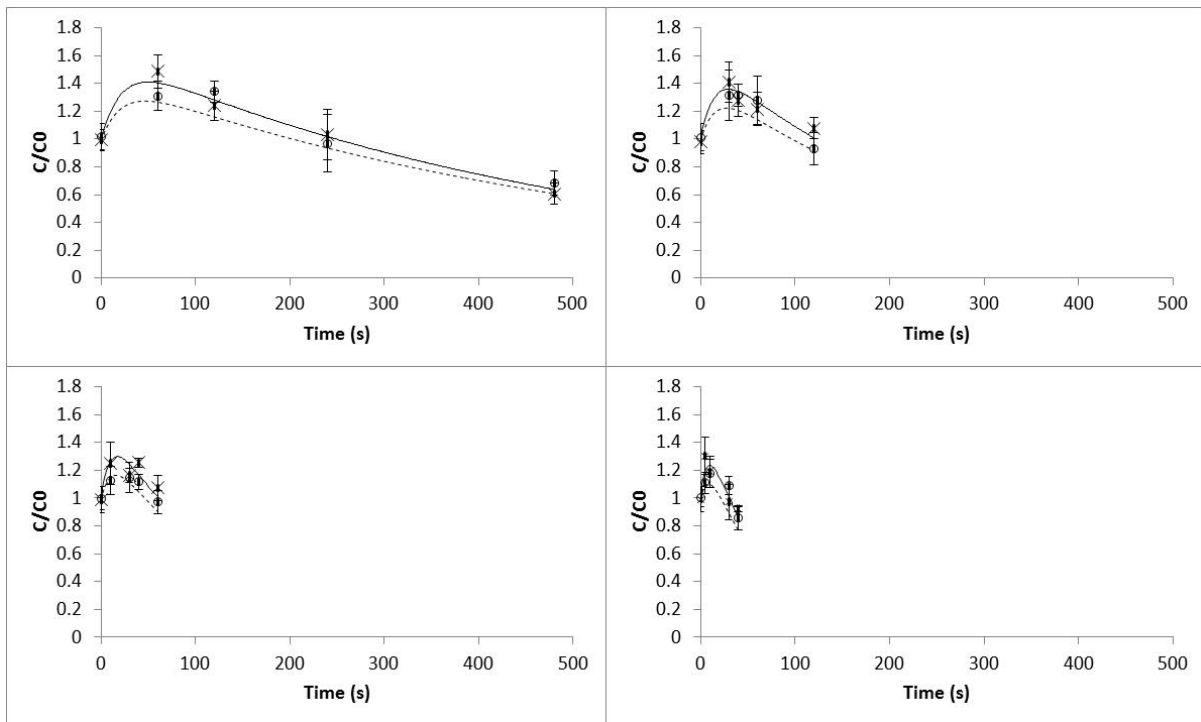


Figure 3: Effect of temperature and UV-C light over time on colour a parameter in Portuguese cabbage in the temperature range 80-95 °C: (x) experimental values of a parameter with heat blanching processes; (—) model predicted values for heat blanching processes; (o) experimental values of colour a parameter with combined UV-C light and heat blanching processes; (---) model predicted values for with combined UV-C light and heat blanching processes. Bars represent mean \pm standard deviation ($n=15$)

experimental data for L and b parameters and a parameter, respectively (Table 1) and the temperature dependence followed the Arrhenius behaviour. In all cases, normality and randomness of residuals were verified, and the coefficient of determination, R^2 , was higher than 0.95. No significant differences ($p>0.05$) were detected between the estimated reaction rates and activation energies at the reference temperature (87.5 °C) for both treatments. The activation energy values for colour changes varied between 63.96 and 155.33 kJ mol^{-1} for L and a parameters in the UV-C and heat blanching treatment, respectively. The high activation energies obtained revealed that Portuguese cabbage is sensitive to the temperatures applied during the blanching process. Cruz, Vieira, and Silva (2007) presented higher activation energies for Lab parameters

($E_{aL}=235.83\pm 89.32 \text{ kJ mol}^{-1}$, $E_{aa}=187.70\pm 160 \text{ kJ mol}^{-1}$ and $E_{ab}=330.62\pm 128.16 \text{ kJ mol}^{-1}$) in thermosonicated watercress leaves. Recently, Aamir, Ovissipour, Rasco, Tang, and Sablani (2014) determined in blanched spinach leaves (65-85 °C) an activation energy value of 117.7 kJ mol^{-1} for colour a parameter. In another study, Gonçalves, Pinheiro, Abreu, Brandão, and Silva (2010), in blanched carrot, reported values of $E_{aL}= 187.03\pm 13.60 \text{ kJ mol}^{-1}$, $E_{aa}= 186.39\pm 10.33 \text{ kJ mol}^{-1}$ and $E_{ab}= 231.16\pm 17.01 \text{ kJ mol}^{-1}$.

3.3 Texture changes

Raw Portuguese cabbage presented values of firmness of $168.17\pm 35.42 \text{ g}$. Figure 5 shows the effect of heat and UV-C light followed by heat in

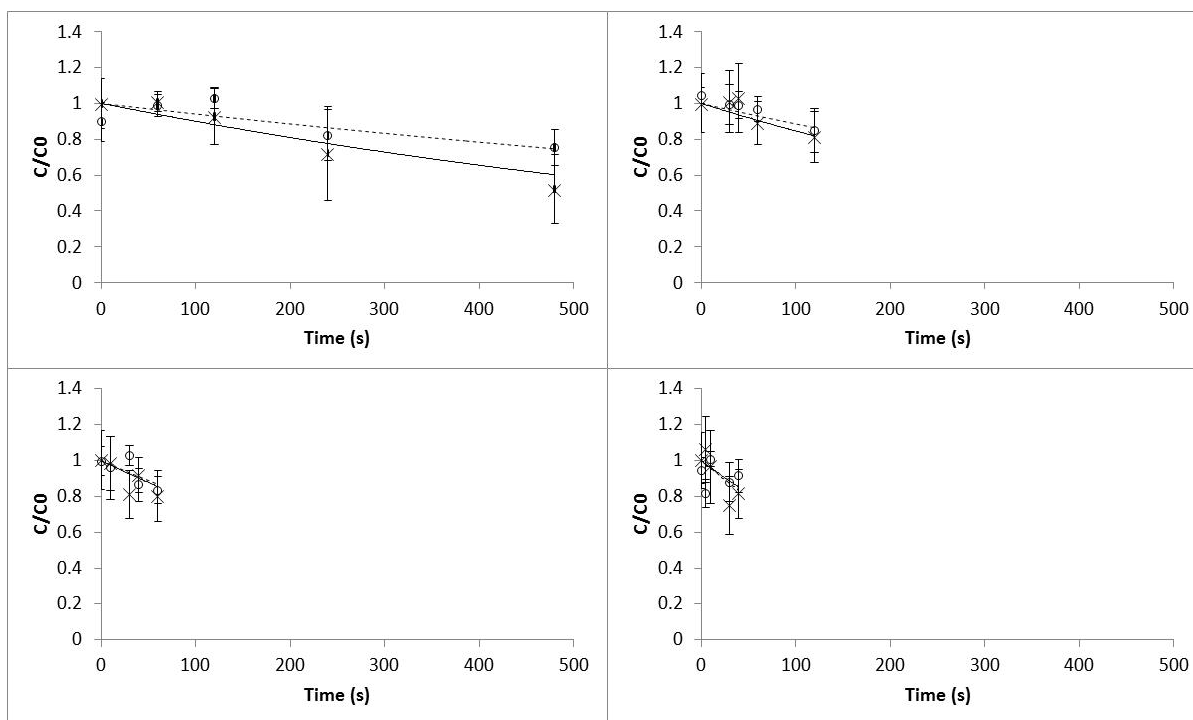


Figure 4: Effect of temperature and UV-C light over time on colour b parameter in Portuguese cabbage in the temperature range 80-95 °C: (x) experimental values of b parameter with heat blanching processes; (—) model predicted values for heat blanching processes; (o) experimental values of colour b parameter with combined UV-C light and heat blanching processes; (---) model predicted values for with combined UV-C light and heat blanching processes. Bars represent mean \pm standard deviation ($n=15$)

the firmness of the Portuguese cabbage leaves. The firmness of the cabbage leaves showed no significant differences between blanching treatments (ANOVA, $p>0.05$). Texture changes in Portuguese cabbage leaves also showed two distinct mechanisms. First, at short processing times (5-60 s and 80-95 °C), the Portuguese cabbage leaves increased in firmness to 130-140% compared to initial values. Then, the samples became softer when the processing time increased. Ni, Lin, and Barrett (2005) also reported that the firmness of a variety of vegetables (broccoli, Pak choy, Chinese cabbage, cabbage, green peppers, sugar snap peas and carrots) blanched at different temperatures (55, 60, 65, 70, 75, and 80 °C) and times of exposure (5, 15, 30, 45 and 60 min) showed between 80 and almost 200% increase in firmness compared to the blanched control (100

°C and 3 min.). Moreover, AnzaldúaMorales, Quintero, and Balandran (1996) reported a pronounced increase in the firmness of lentils in the initial stage of blanching at 80 °C and 85 °C compared to blanching at 90 and 95 °C. During thermal processing, a range of enzymatic and chemical reactions occur, which alter the texture of processed vegetables. The chemical changes, such as solubilisation and depolymerisation of pectic polysaccharides, affect the constituents of the cell wall and middle lamella, thereby resulting in a major change in the firmness of vegetables (Nisha, Singhal, & Pandit, 2006). This increase in firmness may be the result of the availability of calcium ions for cross-link formation with adjacent pectin molecules (Latorre, de Escalada Plá, Rojas, & Gerschenson, 2013) and also the role of pectin methyl esterase (PME), in

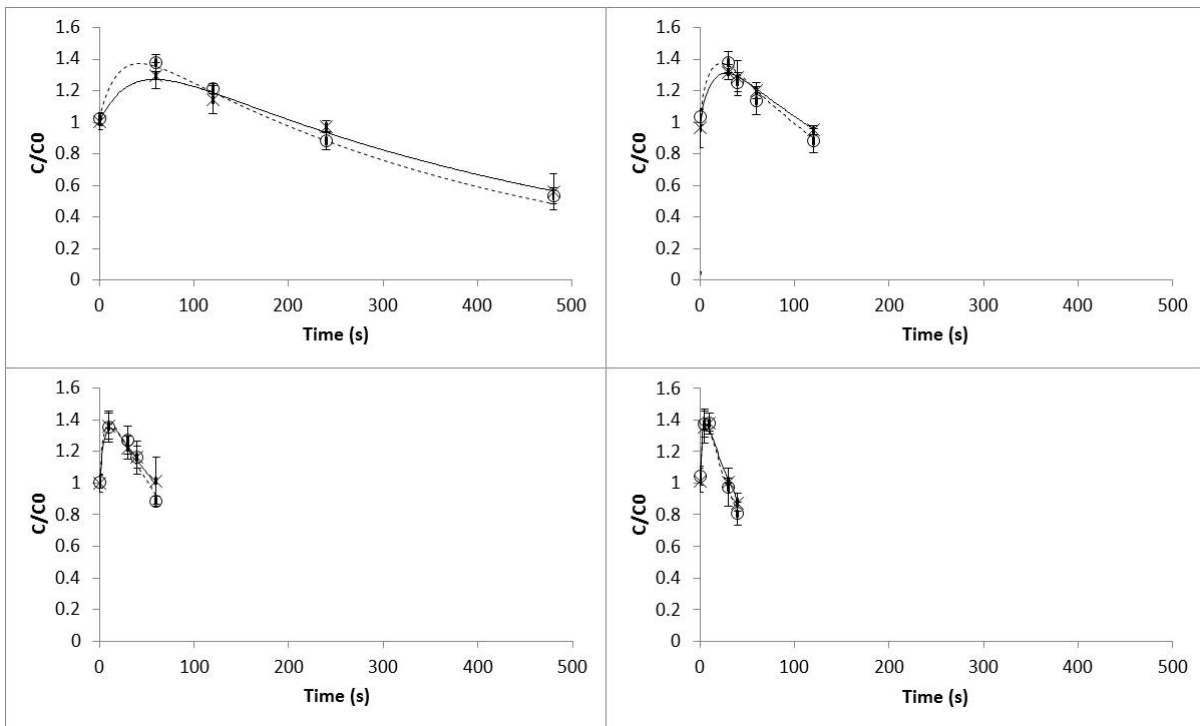


Figure 5: Effect of temperature and UV-C light over time on texture (firmness) in Portuguese cabbage in the temperature range 80-95 °C: (x) experimental values of texture (firmness) with heat blanching processes; (—) model predicted values for heat blanching processes; (o) experimental values of texture (firmness) with combined UV-C light and heat blanching processes; (---) model predicted values for with combined UV-C light and heat blanching processes. Bars represent mean \pm standard deviation ($n=3$)

hydrolyzing the methyl ester linkages in pectin molecules, releasing methanol and free galacturonic acid moieties. The resulting free carboxyl groups may then form cross-links between pectin polymers through salt-bridge formation with divalent cations (particularly Ca^{2+}) naturally present in the tissues (Puri, Solomos, & Kramer, 1982; Ni et al., 2005). At higher temperatures the permeability of membranes is increased, resulting in passive efflux of solutes and an irreversible reduction of cell turgor well before membranes are completely destroyed (De Belie, Herpich, & De Baerdemaeker, 2000).

A two fractions first-order model fitted well the experimental data (Table 1) and the temperature dependence of firmness also followed the Arrhenius behaviour. The estimated reaction rates and activation energies at the reference temperature

(87.5 °C) for both treatments were very similar (R^2 and adjusted R^2 presented values of 0.99 for both treatments). The activation energy values for texture changes, for each fraction, varied between 135.68 and 186.86 kJ mol^{-1} . Jaiswal et al. (2012) reported a lower activation energy value of 33.88 kJ mol^{-1} for Irish York cabbage blanched at temperatures varying between 80-100 °C during 2-14 min. Huang and Bourne (1983) reported activation energies ranging from 21.35 to 146.50 kJ mol^{-1} for different vegetables. (Anzaldúa-Morales et al., 1996) reported activation energy values of 107.50 kJ mol^{-1} , 147.40 kJ mol^{-1} and 210.50 kJ mol^{-1} for the texture of blanched (80-95 °C) white beans, chick peas and broad beans, respectively. Lau et al. (2000) studied the kinetics of textural changes in green asparagus during thermal treatments (70-98 °C) and reported an

activation energy value of 100.6 kJ mol⁻¹. In another study Gonçalves et al. (2007) reported an activation energy value of 72.21 kJ mol⁻¹ in blanched pumpkin (75-95 °C). Vu et al. (2004) reported an activation energy of 117.6 kJ mol⁻¹ in blanched carrot (80-110 °C). Possibly, these variations are related to the differences in the raw material, modelling methodologies and the temperature ranges used in each study.

In commercial scenarios, to obtain vegetables that maintain optimum quality during storage, a reduction of 90% of the POD activity after blanching is recommended (Bahçeci, Serpen, Gokmen, & Acar, 2005). In the present study, the UV-C light followed by heat treatment, for example at 80 °C, required only 1.30 min to reduce 90% of the POD activity, while maintaining 98% of lightness and 92% of yellowness and increasing green colour (130%) and the firmness (125%) of the Portuguese cabbage leaves. On the other hand, the traditional heat blanching treatment at 80 °C requires 7.4 min to reduce 90% of the enzyme activity. This treatment reduces lightness, greenness, yellowness and firmness to 92%, 68%, 62% and 61% of initial values, respectively. Therefore, for the inactivation of this enzyme, the alternative treatment may be used to minimize colour and texture changes, since processing times can be reduced. It should be also remarked that instead of promoting colour and texture degradation, the applied treatment at lower short processing times led to greener and firmer Portuguese cabbage leaves.

4 Conclusions

The combination of UV-C light followed by heat blanching treatment at 80 and 85 °C was shown to significantly shorten the treatment time needed to inactivate POD in Portuguese cabbage. Thus, it can be considered effective to be used as a hurdle prior to a heat blanching treatment and provide a practical alternative to the traditional blanching treatments for inactivating POD in Portuguese cabbage. Moreover, this combined treatment was revealed to be a better blanching process due to the good quality retention in the treated produce. The present findings will help optimize the Portuguese cabbage

blanching conditions by heat blanching combined with UV-C light treatment.

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