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ARTICLE

Degradation Kinetics of Peroxidase Enzyme, Phenolic Content, and Physical and Sensorial Characteristics in Broccoli (*Brassica oleracea* L. ssp. *Italica*) during Blanching

Elsa M. Gonçalves,[†] Joaquina Pinheiro,[†] Carla Alegria,[†] Marta Abreu,[†] Teresa R. S. Brandão,[‡] and Cristina L. M. Silva*[,‡]

[†]Departamento de Tecnologia das Indústrias Alimentares, Instituto Nacional de Engenharia, Tecnologia e Inovação, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal, and [‡]Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

The effects of water blanching treatment on peroxidase inactivation, total phenolic content, color parameters [$-a^*/b^*$ and hue ($h°^*$)], texture (maximum shear force), and sensory attributes (color and texture, evaluated by a trained panel) of broccoli (*Brassica oleracea* L. ssp. *Italica*) were studied at five temperatures (70, 75, 80, 85, and 90 °C). Experimental results showed that all studied broccoli quality parameters suffered significative changes due to blanching treatments. The vegetal total phenolic content showed a marked decline. Degradation on objective color and texture measurements and alterations in sensorial attributes were detected. Correlations between sensory and instrumental measurements have been found. Under the conditions 70 °C and 6.5 min or 90 °C and 0.4 min, 90% of the initial peroxidase activity was reduced. At these conditions, no significant alterations were detected by panelists, and a small amount of phenolic content was lost (ca. 16 and 10%, respectively). The peroxidase inactivation and phenolic content degradation were found to follow first-order reaction models. The zero-order reaction model showed a good fit to the broccoli color ($-a^*/b^*$ and $h°^*$), texture, and sensory parameters changes. The temperature effect was well-described by the Arrhenius law.

KEYWORDS: Broccoli; blanching; quality parameters; kinetics modeling

INTRODUCTION

Vegetables contribute to humans essential minerals, nutrients, and energy. The regular consumption of vegetables can play an important role in the prevention of certain diseases, such as cancer and cardiovascular problems (*1*). Broccoli (*Brassica oleracea* L. ssp. *Italica*) is the one of the most nutritious of the cole crops in vitamin content, calcium, and iron and is a good source of health-promoting compounds, such as phenolics (*2*) and glucosinolates (*3*), which are a large group of sulfur-rich, anionic, natural products. The anticancer advantage of broccoli has been recently reported (*4*). However, vegetables not only form an essential part of a well-balanced diet, but their sensory attributes, namely, texture and color, make them important in human appetite.

Fresh vegetables are perishable products, and for this reason, they must be preserved to maintain their property levels or their natural benefits. Fresh broccoli has a limited shelf life, less than 3 days at 20 °C (*5*). Freezing is a widely used technology to preserve this vegetable for long periods. However, in general, each step of the freezing process, especially the blanching operation, affects the vegetable characteristics, which may be desirable or not. Thus, understanding the effects of each process step on product quality is critical for food-processing optimization.

Water blanching is a thermal pretreatment necessary to improve the quality of frozen vegetables, since it inactivates enzymes responsible for quality degradation and destroys vegetative microbial cells, allowing stabilization and product quality retention during storage (*6*). However, the impact of heat on quality attributes of vegetables is complex and not always beneficial. Industrial blanching processes involve temperatures ranging from 70 to 95 °C and times usually not higher than 10 min (*7*). In general, heat applied to green vegetables degrades chlorophyll pigments, resulting in a change of greenness visual color (*8*), and produces structural deformation on vegetal tissue, promoting a gradual decrease of product texture (*9*). Additionally, bioactive compounds, such as vitamins, are affected by blanching due to thermal degradation or leaching phenomena into the heating medium (*10*).

For this reason, recent developments and advances in low-thermal technologies have been studied. As an example, the development of termosonication and ohmic technologies appears to offer some benefits (*11*, *12*). However, none of these technologies have yet been applied in the processing of vegetables for various reasons. Therefore, it is clear that the current trend is to optimize the blanching process and consequently to minimize the

*To whom correspondence should be addressed. Tel: +351-22-5580058. Fax: +351-22-5090351. E-mail: clsilva@esb.ucp.pt.

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Table 1. Published Kinetic Parameters for POD Thermal Inactivation

ref	product	temperature range (°C)	kinetic parameters			kinetic model
			k (min^{-1})	T_{ref} (°C)	E_a (kJ mol^{-1})	
(*11*)	watercress	40−92.5	18_{HLF} 0.24_{HRF}	84.6	421_{HLF} 352_{HRF}	Arrhenius biphasic
(*15*)	green beans	70−95	2.15	85	99.1	Arrhenius first-order
(*16*)	broccoli	50−100			$388_{acidic\ form}$ $189_{neutral\ form}$ $269_{basic\ form}$	Arrhenius first-order
(*7*)	broccoli	70−95	63.5[a] 2277[a]	80	75_{HLF} 58_{HRF}	Arrhenius biphasic first-order
(*17*)	carrot	35−75	33.0[a] 2000[a]	75	90_{HLF} 148_{HRF}	Arrhenius biphasic first-order
(*18*)	pumpkin	75−95	0.3	85	86	Arrhenius first-order
(*19*)	butternut squash	60−90	24.9 0.07	65	14_{HLF} 158_{HRF}	Arrhenius biphasic first-order ($T < 70$ °C)
			8.6	85	150	Arrhenius monophasic first-order ($T > 70$ °C)

[a] Value estimated.

thermal impact on vegetable quality. The optimization of the blanching processes requires not only the knowledge of the conditions that lead to enzyme inactivation but also the relationship between product quality parameters, nutritional and/or sensory, and thermal process time−temperature.

Peroxidase (POD), an enzyme belonging to the oxidoreductase class, is the most thermally resistant enzyme in vegetables, and for this reason, it is usually used as a biological indicator of blanching effectiveness (*13*, *14*). The inactivation of POD has been studied on different vegetables. **Table 1** reviews recent kinetics data on POD thermal inactivation in some vegetables. The majority of the published work reports biphasic first-order degradation reaction kinetics; however, other inactivation models based on different mechanisms were also applied.

Phenolic compounds have been categorized into different groups such as flavonoids, tannins, phenolic acids, and coumarins (*20*) and constitute one of the most important groups of natural antioxidants, because of their diversity and extensive distribution. Data on total phenolic content of processed green vegetables are recent and very limited (*21*). Some studies reported that vegetal phenolic content is influenced by technological treatments, thus affecting their stability, biosynthesis, and degradation (*22*, *23*). No published data on thermal phenolic degradation kinetics have been found in the literature.

Color and texture are important quality attributes in determining vegetable acceptability. Several researchers have published work on modeling of thermal degradation kinetics of color and texture in different vegetables (*24*−*26*). Only Weemaes et al. (*27*) described the thermal degradation kinetics of green color ($-a$ value) in broccoli juice, applying a two-step fractional conversion model. However, there is a lack of studies on thermal degradation kinetics on broccoli texture, probably due to its structural heterogeneity with consequent high difficulty of evaluation.

Physical instrumental analyses are the usual methods used to assess color and texture of processed vegetables. However, sensory analysis, involving a taste panel, provides a sophisticated tool to obtain a realistic description of the product's consumer perception. Relationships between descriptive sensory attributes or consumer preferences and instrumental evaluation should be determined in food products (*28*). Only two kinetic studies were found dealing with modeling subjective evaluation changes (*29*, *30*). The need to have a high performance of the individual judges that constitute the taste panel may be a cause for the few studies carried out.

The present study was undertaken to investigate the kinetics of POD inactivation, total phenolic content, color ($-a^*/b^*$ and $h°^*$), firmness (maximum shear force), and sensory characteristics (texture and color) degradations of thermally processed broccoli (*B. oleracea* L.), under five blanching isothermal conditions. The aim was to model all of these quality parameters for the optimization of the blanched broccoli by immersion in hot water.

MATERIALS AND METHODS

Raw Material and Processing Conditions. Fresh broccoli (*B. oleracea* L. spp. *Italica* cv. Marathon) was obtained directly from a cultivated area in Torres Vedras, Portugal. Broccoli inflorescences were cleaned of leaves and divided into florets about 5 cm in diameter, cutting stems 2 cm below the lowest ramification. For heat treatments, samples were immersed in a thermostatic water bath (±1 °C) at five temperatures, in the range of 70−90 °C, and times, until 40 min (not in sequence); the ratio sample weight/water volume was ca. 500 g of fresh product/50 L. During the heat treatments, the temperatures of the water and samples were monitored by means of thermocouples (type T thin thermocouple, 1.2 mm diameter, embedded in a stainless steel hypodermic needle, Ellab, Denmark, with an accuracy of ±2 °C). After they were cooled in an ice water bath (2 min), excess of moisture was removed before any further analysis. Each experiment was replicated twice. A representative sample of the whole batch of the raw material was used as a reference.

POD Enzyme Assay. The POD activity was assessed spectrophotometrically, according to the method described by Bifani et al. (*15*). Raw and blanched broccoli samples (20 g each) were weighed into 100 mL of 1 M sodium chloride solution. The samples were homogenized with a blender at 4 °C for 2 min. The homogenate was centrifuged in polypropylene tubes at 5856*g*, using a Sorvall Instruments RC5C centrifuge (Dupont, Wilminton, United States), at 4 °C for 15 min. The slurry was filtered using 1.2 μm membrane filters (Whatman). The filtrate was mixed with guaiacol and H_2O_2 as substrates. The absorbance increase at 470 nm was recorded using an UV/vis, ATI Unicam spectrophotometer (Unicom Limited, Cambridge, United Kingdon). The definition used for 1 unit of enzyme activity was the amount of enzyme that produced a change in absorbance of 1.0 per min per mL of extract sample, under the assay conditions. The enzymatic activity is expressed as activity units/g fresh tissue. The results of the residual activity were normalized in relation to specific activity observed in the raw product. The analyses were carried out in duplicate. The initial enzyme activity was determined from five samples of fresh product.

Total Phenolic Content Assay. Raw and processed vegetable samples (duplicates) were homogenized with 70% ethanol (1:1, w/v), using a Yellow line DI 25 basic polytron (IKA-Labortechnik, Stauten, Germany). The amount of total phenolic content was determined using Folin−Ciocalteu reagent, as described by Singleton et al. (*31*). After centrifugation (43140*g* × 20 min × 4 °C, Sorvall RC-5, rotor SS34), the mixture was placed in a water bath (45 °C for 15 min), and the absorbance was measured at 765 nm in the ATI Unicam spectrophotometer, using gallic acid as the standard. Results were expressed as milligram gallic acid equivalents (mGAE) per 100 g fw. Six measurements per sample were obtained.

Color Evaluation. For the color determinations, 30 g of broccoli was triturated and analyzed using a tristimulus colorimeter (Colorgard System/05 Gardner port size). The colorimeter was calibrated against standard white and black tiles. Measurements were performed in the CIE $L^*a^*b^*$ system, using an illuminate C. The lightness value, L^*, indicates how dark/light the sample is (varying from 0, black, to 100, white), a^* is a measure of greenness/redness (varying from -60 to $+60$), and b^* is the grade of blueness/yellowness (also varying from -60 to $+60$). To describe the color behavior of broccoli during blanching treatments, the ratio of ($-a^*/b^*$) and the hue angle, $h°^*$ (0−360°) (obtained by $\tan^{-1} b^*/a^*$, expressing the characteristic/dominant color), were used. Data were obtained from 30 measurements on each sample.

Texture Evaluation. Texture was determined by a shearing test performed with a Kramer cell (standard cell with 10 blades), using a TA.HDi. texture analyzer (Stable Micro-System Ltd., Godalming, United Kingdom). Thirty grams of broccoli pieces was placed randomly in the base of the cell, and the maximum shear force (N) values were recorded and used as indicators of textural parameter. The test speed was 8 mm s^{-1}, and the full-scale load was 500 N. Texture measurements were determined on the basis of 12 replications for each time−temperature combination.

Sensorial Assessment. For sensory evaluation, a quantitative descriptive analysis was used according to Van Loey et al. (*30*). Ten trained panelists, who met the basic requirements of sensory sensitivity according to ISO 8586-1 (*32*) in adequate conditions conforming to ISO 13299 (*33*), identified and discriminated color and texture sensory attributes of blanched broccoli.

Two descriptors were employed to grade the quality in terms of color and texture, using a 10 cm unstructured scale, labeled from 1.0 to 9.0, corresponding to very good and very poor, respectively. Samples (±20 g) of broccoli were served in plastic dinner plates coded with three random digits and served in randomized order. The panelists were asked to mark the perceived intensity of the attribute by drawing a vertical line on the scale and writing the code. During each session, the assessor had to analyze five processed samples in color and texture quality attributes at the same temperature and different times. A duplicate was given at each session just to check the panel performance, and the data arising from this duplicate sample were not used for the final analysis. The duplication of sample was also randomized for each panelist. It was found that there was no significant difference among the duplicates, indicating good panel consistency ($P \leq 0.05$). From the length on the scale where the intensity of the attributes was marked, the intensity scores were tabulated. The mean scores for intensity were calculated.

Data Analysis. An analysis of variance (two-way ANOVA) was performed to determine significant effects on experimental data due to blanching time−temperature conditions. Reaction rate constants of broccoli POD inactivation; phenolic content, color ($-a^*/b^*$ and $h°^*$), and texture (maximum force) changes; and sensorial analysis were estimated by nonlinear regression analysis, fitting zero- or first-order kinetic models, eqs 1 and 2 (depending on the parameter considered), to isothermal experimental data.

$$P = P_0 - k_T t \qquad (1)$$

$$\frac{P}{P_0} = e^{-k_T t} \qquad (2)$$

In the previous equations, P is the evaluated parameter, the subindex 0 indicates the initial value, t is the heating time, and k is the rate constant at temperature T.

The temperature effect on rate constants was described by the Arrhenius law (eq 3):

$$k = k_{ref} \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \qquad (3)$$

where k is the reaction rate constant, k_{ref} is the reaction rate constant at a finite reference temperature (T_{ref}), E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature. The reference temperature used was the average value of the range considered (i.e., T_{ref} = 80 °C), aiming at improving parameters estimation.

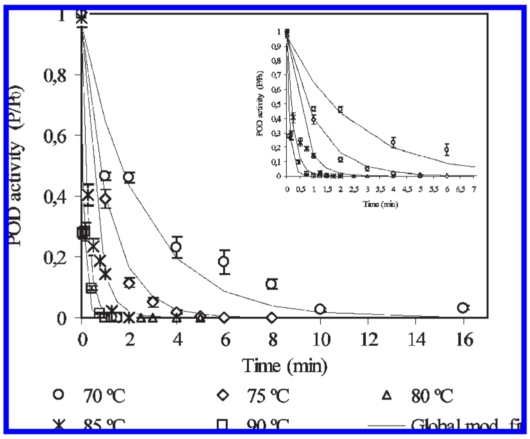

Figure 1. Broccoli POD inactivation during the blanching process. The inset depicts the initial phase of inactivation.

The $k_{80\,°C}$ and the activation energy were estimated directly from experimental data in one step (quality factor vs time, at all temperatures), by performing a global nonlinear regression analysis, merging the Arrhenius equation and the kinetic model considered (*34*, *35*).

The parameters' precision was evaluated by confidence intervals at 95%, and the quality of the regression was assessed by the coefficient of determination (R^2) and randomness and normality of residuals (*36*), thus allowing best fit model parameters. Statistica version 8.0 software (*37*) was used for all regression analysis procedures (using least-squares estimation and Levenverg−-Marquart method, for minimizing the sum of squares of the deviations between the experimental values and the ones predicted by the mathematical model).

RESULTS AND DISCUSSION

Thermal Inactivation of POD. The average specific activity of the enzyme in raw broccoli was 688.5 ± 73.8 U/g. The enzyme activity was significantly affected by the heat treatment intensity. Similar results were obtained by Murcia et al. (*38*), Muftügil (*39*), and Brevwer et al. (*40*). **Figure 1** shows the residual enzyme activity as a function of heating time. The values were normalized in relation to the specific activity observed in the raw product (i.e., P/P_0). The POD inactivation in broccoli has been reported to follow a first-order model or biphasic first-order model (*7*, *16*). Experimental results obtained in this work were also satisfactorily described by an Arrhenius first-order kinetic model (model fit obtained by one-step regression analysis is also included in **Figure 1**). The quality of the model fit was assessed by analyses of the residuals (i.e., normality and randomness were confirmed). The value of R^2 was 0.98. Estimated kinetic parameters and confidence intervals at 95% are in **Table 2**. The estimated E_a value is higher as compared with the value found for broccoli by Morales-Blancas et al. (*7*). Differences could be due to cultivars or measurement method. Published data on POD kinetic values have shown a wide variation (**Table 1**), depending on the vegetable and enzyme distribution on product and different isoenzymes considered (*16*). For optimum quality retention of vegetables during frozen storage, a reduction of 90% of the initial POD activity, after the blanching process (*41*), is recommended. In the case of blanched broccoli at 70, 75, 80, 85, and 90 °C, this target reduction was obtained, respectively, after ca. 6.5, 2.7, 1.2, 0.7, and 0.4 min of the thermal treatment. The rapid POD activity losses during these blanching treatments, especially at 90 °C and 0.4 min, may be due to the small size of the pieces involved. Normally, to promote less damage to the vegetables quality, a high-temperature−short time (HTST) treatment is recommend.

Table 2. Kinetic Parameters, and Corresponding Confidence Intervals at 95%, of Broccoli POD Inactivation and Quality Parameters Deterioration Due to Thermal Treatment

		kinetic parameters		
quality factor (kinetic model)		P_0	$k_{80\,°C}$ (min^{-1})	E_a (kJ mol^{-1})
POD inactivation (Arrhenius first-order; eqs 2 and 3)			0.032 ± 0.002	159.4 ± 5.8
total phenolic content (Arrhenius first-order; eqs 2 and 3)		54.9 ± 4.7	0.03 ± 0.004	75.4 ± 12.3
color (Arrhenius zero-order; eqs 1 and 3)	$-a^*/b^*$	−0.50 ± 0.01	0.005 ± 0.0004	28.5 ± 7.7
	$h°^*$	116.6 ± 0.4	0.2 ± 0.02	29.7 ± 7.6
texture (Arrhenius zero-order; eqs 1 and 3)	maximum shear force (N)	3173.4 ± 41.3	38.8 ± 3.6	158.3 ± 9.2
sensorial evaluation (Arrhenius zero-order; eqs 1 and 3)	color	2.5 ± 0.8	0.2 ± 0.07	69.2 ± 3.5
	texture	1.4 ± 0.5	0.2 ± 0.04	84.2 ± 17.9

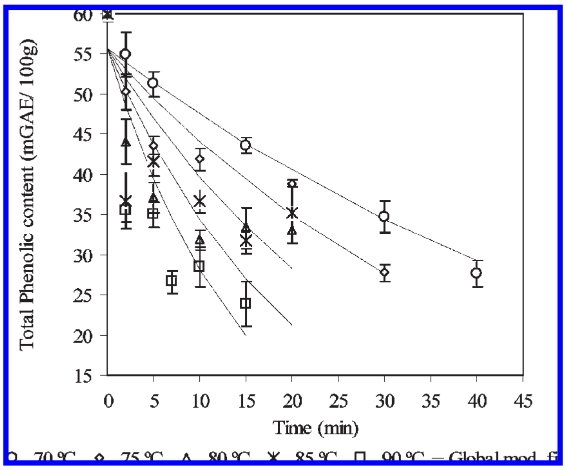

Figure 2. Blanching effect on the total phenolic content of broccoli.

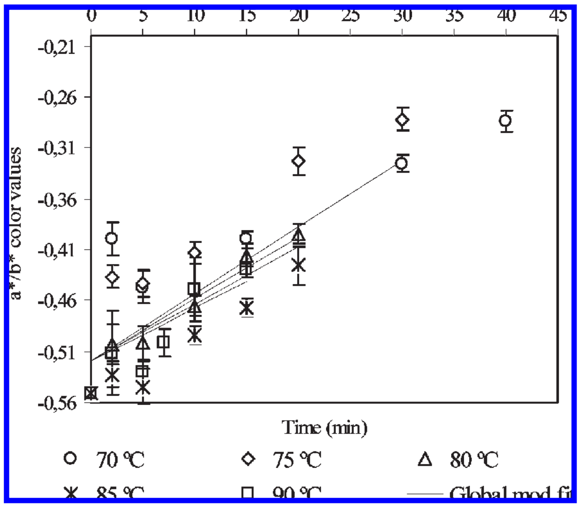

Figure 3. Broccoli color $-a^*/b^*$ values during the blanching process.

However, some studies have revealed that residual, or reactive, POD can occur and cause significant deterioration when these treatments were used (*38*). This fact poses a problem in HTST treatments of vegetables that are, for example, subsequently frozen.

Losses in Total Phenolic Contents. Fresh broccoli florets contained 59.9 ± 1.0 mGAE/100 g of fw. This value is similar to the reported by Leja et al. (*42*), 56.2 mg/100 g fw, and higher than what was reported by Zhang and Hamauzu (*43*), ca. 35 mg/100 g fw. However, Turkmen et al. (*44*) reported that raw broccoli floret contained 1204.3 mg/100 g DM of total phenolics. These differences in raw broccoli phenolic concentration may be due to differences in the maturity stage, agricultural conditions, varieties, and cultivars or postharvest practices (*21*, *42*, *45*).

Blanching caused a significant decrease of broccoli total phenolic content, probably due to thermal degradation and leaching phenomena to water. Total phenolic values decreased with treatment intensity (**Figure 2**). Similar results were reported by Oboh and Akindahunsi (*46*) in different green leafy vegetables.

An Arrhenius first-order kinetics model (eqs 2 and 3) was used to model the thermal degradation of total phenolic content (**Figure 2**). The activation energy and the reaction rate at a reference temperature (80 °C) are reported in **Table 2**. The value of regression coefficient was 0.83. Moreover, the activation energy value (75.4 kJ mol^{-1}) is in the range normally expected for nutrients nonenzymatic degradation reactions (*10*).

If the two extreme proposed conditions to inactivate 90% of POD initial activity (70 °C for 6.5 min and 90 °C for 0.4 min) were considered, total phenolic content would suffer a small variation, only ca. 16 and 10%, respectively, in relation to raw product.

Color Changes. The nonheated broccoli (the reference raw sample) exhibited a light green color, corresponding to the following average values of the color coordinates: L^* = 47.6 ± 0.5, a^* = −10.0 ± 0.5, b^* = 20.1 ± 0.9, and $h°^*$ = 116.6 ± 0.6. The $-a^*/b^*$ values increased significantly ($P \leq 0.05$) with blanching time and temperature (**Figure 3**), and the $h°^*$ values decrease significantly ($P \leq 0.05$) with intensity of treatment (data not showed). Broccoli color changes from a bright green to a brownish olive green. These modifications may be explained by conversion of chlorophylls into pheophytins and pyropheophytins promoted by heat or coloring compounds lixiviation into water, as stated by Tijskens et al. (*25*).

An Arrhenius zero-order model (eqs 1 and 3) was successfully fitted to experimental data of $-a^*/b^*$ and $h°^*$ (estimated parameters and regression analysis results are included in **Table 2**). Experimental $-a^*/b^*$ data and model fits can be observed in **Figure 3**. In all cases, normality and randomness of residuals were verified, and the coefficient of determination, R^2, was satisfactorily high, 0.84 and 0.85 for $-a^*/b^*$ and $h°^*$, respectively. Obtained activation energies are considerably low as compared with results obtained for other green vegetables (*24*, *47*), which is indicative that broccoli kinetics of color change is not sensitive to blanching temperature. If the extreme proposed blanching conditions (70 °C for 6.5 min or 90 °C for 0.4 min) were used, color factors would suffer, respectively, unperceived with a 5% change in relation to the raw product.

Texture Changes. Figure 4 presents results for the texture parameter (maximum shear force) of broccoli submitted to thermal treatments. The maximum shear force average value of raw broccoli was 3209.8 ± 110.4 N.

The analysis of variance demonstrated that the texture was significantly affected by heating temperature and process time. However, as it can be observed in **Figure 4**, under the experimental conditions, 70 °C and 40 min and 90 °C and 15 min,

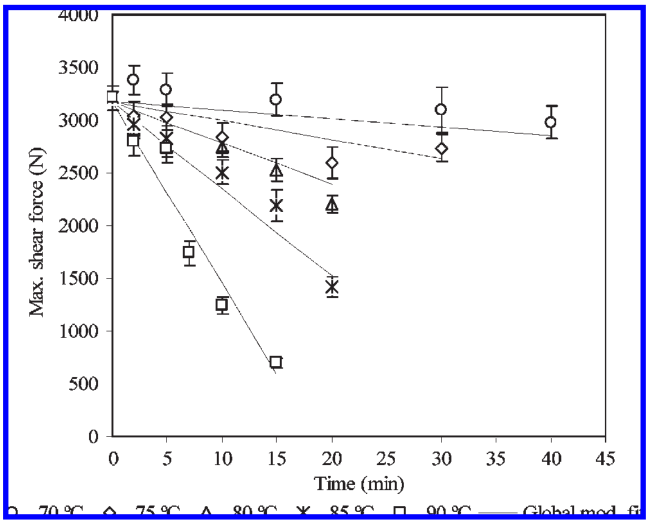

Figure 4. Broccoli maximum shear force (N) values during the blanching process.

texture showed to be more retained at lower temperature and longer time (ca. 7 and 78%, respectively). This effect can be attributed to the fact that PME enzyme increases its activity at temperatures between 50 and 70 °C; thus, pectic substances are demethoxylated, giving rise to the formation of calcium and magnesium pectates (*48*).

A zero-order kinetic model with Arrhenius temperature dependence (eqs 1 and 3) fitted well the experimental data for firmness values (R^2 = 0.95) (see **Figure 4**). The obtained reaction rate constant at 80 °C and activation energy for texture parameter were, respectively, 38.8 min^{-1} and 158.3 kJ mol^{-1}. Activation energy values of 100.6 kJ mol^{-1} for green asparagus and 139.4 kJ mol^{-1} for garlic were reported by Lau et al. (*24*) and Rejano et al. (*26*), respectively. Comparing results, the value obtained is higher, which is indicative that broccoli texture is more sensitive to the blanching process temperature. This study revealed that broccoli texture was the most temperature sensitive (higher E_a values).

Changes in Sensory Attributes. The effects of blanching on broccoli color and texture sensory evaluated quality attributes (average value ± standard deviation) are presented in **Figure 5a**,**b**, respectively. It can be observed that those attributes suffered significant changes due to blanching. The panelist's evaluation indicated that samples suffered a notable loss of green color and their firmness decreased.

Both sensory parameters degradation followed zero-order kinetics with heating treatment. Estimated activation energies, rate constants at the reference temperature of 80 °C, and corresponding 95% confidence intervals are included in **Table 2**. Published activation energies data on color and hardness sensorial evaluated for peas and white beans (*30*) were slightly higher than the values reported in this study. For example, for white beans hardness, an E_a value of 97 kJ mol^{-1} was reported. In addition, significant correlations were found between physical measurements (maximum force and $-a^*/b^*$ color parameter) and sensory perceived changes of texture and color. The correlation coefficients were 0.95 and 0.78, respectively ($P \leq 0.05$). No perceived changes were observed by panelists if any the proposed conditions to inactivate 90% of the initial activity (70 °C and 40 min or 90 °C and 15 min) were applied to blanched broccoli.

POD inactivation and total phenolic content degradation in blanched broccoli follows first-order inactivation kinetics. The other analyzed quality factors, color, texture, and sensorial attributes (color and texture), were well-described by a zero-order

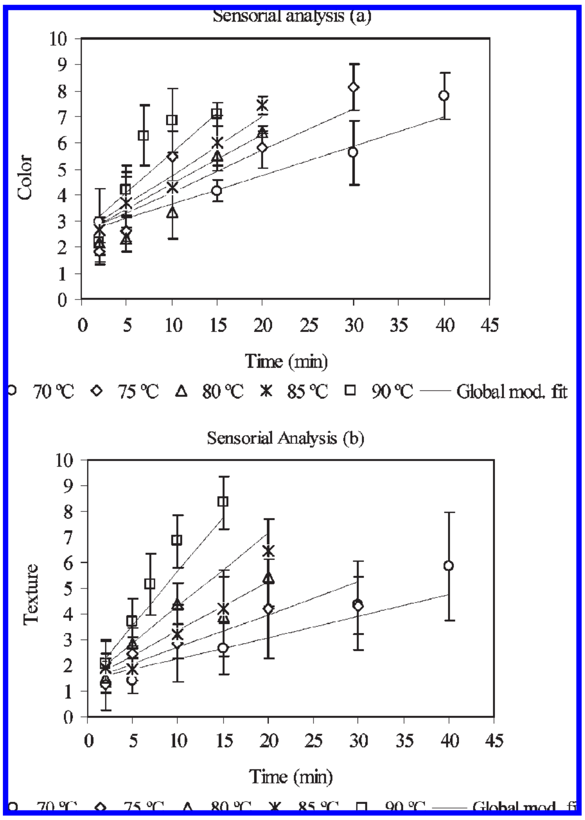

Figure 5. Blanching effect on broccoli sensory properties: (**a**) color and (**b**) texture.

model. Because food quality is highly valued by consumers, changes may be understood and controlled during processes. The blanching conditions (70 °C and 6.5 min or 90 °C and 0.4 min) are recommended to inactivate 90% of POD activity and avoid detrimental phenomena in broccoli or sensorial losses. However, this study revealed that broccoli texture was the most temperature-sensitive parameter. Thus, attention may be given to texture against other quality parameters for optimizing broccoli thermal processes.

ABBREVIATIONS USED

a^*, CIE color space coordinate (degree of greenness/redness); b^*, CIE color space coordinate (degree of blueness/yellowness); $h°^*$, hue index; E_a, activation energy (J mol^{-1}); fw, fresh weight; k, rate constant (min^{-1}); L^*, CIE color space coordinate (degree of lightness); P, numerical value of quality factor at time t (POD activity, CIE color, texture parameters, sensorial parameters, or total phenolic content); R, universal gas constant (8.314 J mol^{-1} K^{-1}); t, time (min); T, temperature (K); ref, reference value; 0, initial (referring to raw product) value; HLF, heat-labile fraction; HRF, heat-resistant fraction.

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