Biochemical and colour changes of watercress (*Nasturtium officinale* R. Br.) during freezing and frozen storage

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A B S T R A C T

The effects of water blanching, freezing, and frozen storage during 400 days at three different temperatures (−7, −15 and −30 °C), on watercress (*Nasturtium officinale* R. Br.) colour Hunter Lab parameters, chlorophyll degradation, vitamin C content loss and peroxidase (POD) activity were evaluated. The blanching induced significant changes on colour values and chlorophylls and vitamin C contents. POD activity was reduced 85% from its initial value. Freezing did not affect chlorophylls and vitamin C levels, however, promoted significant differences in colour values and POD residual activity. During frozen storage, ascorbic acid (AA) and POD activity degradations followed first-order kinetics, and colour parameters ($L_H \cdot a_H \cdot b_H$, $-a_H/b_H$, $L_H \cdot a_H/b_H$, $L_H/a_H \cdot b_H$ and hue (h^0_H)) were successfully described by zero-order kinetics. The storage temperature effect was successfully described by the Arrhenius law. Chlorophylls and dehydroascorbic acid (DHAA) contents were kept constant during frozen storage.

Introduction

Watercress (*Nasturtium officinale* R. Br.) is an aquatic perennial herb from Cruciferae family, which is native to Europe and an economically important vegetal in Portugal, with wide applications in local cuisine. Raw watercress leaves are used as salad greens, or can be steamed and consumed as a normal processed vegetable. Watercress contains a relatively large amount of vitamins C and provitamin A, folic acid, iodine, iron, protein, and especially calcium and sulphur compounds, which influence its characteristic odour, but also adds to its nutritional benefits (Rose et al., 2000). In addition, watercress has also a history of medicinal use. This vegetable has been the focus of several studies regarding its anticancer properties, mainly due to its high anti-oxidants content (Murphy et al., 2001). However, in spite of its importance, the application of preservation methods, like freezing, for its shelf-life extension is still very limited, and there is a lack of information on this subject.

Freezing is one of the most important methods for retaining vegetables quality during long-term storage. Blanching, as a pre-freezing operation, is a thermal treatment applied to raw vegetables that determines largely the final product quality. Its main objective is the inactivation of the enzymes responsible for deterioration reactions during frozen storage. The intensity of this treatment has to assure the enzymatic inactivation, while minimising the possible negative effects of heat on product quality, such as degradation of texture, and vitamins and colour changes.

During frozen storage, the properties of the vegetables are greatly influenced by storage conditions, especially temperature and time, even at low temperatures, suffering important quality attributes modification as a result of the action of biochemical activity, chemical and physical phenomena (Giannakourou and Taoukis, 2003).

Colour, is one of the most important attributes which affects the consumer perception, and is also an indicator of the vegetable pigment concentration (Francis, 1995). During freezing and frozen storage, the colour of green vegetables suffers modifications due to chlorophylls changes, which can follow chemical and enzymatic pathways. The chemical pathway involves removal of the Mg^{2+} ion from the porphyrin ring via: (i) acidic substitution and/or heat, such as in the conversion of chlorophylls into to pheophytins and (ii) or decarbomethoxylation, such as in the conversion of pheophytins or pheophorbide to pyropheophorbide, respectively. Enzymatic changes are usually due to the action of chlorophyllase on chlorophylls, resulting in chlorophyllides and pheophytins, and then pheophorbides (Heaton et al., 1996). However, other enzymes, like peroxidases, lipases and lipoxygenases, are also associated with the chlorophylls conversion into pheophytins (Buckle and Edwards, 1970).

Ascorbic acid (AA) (or vitamin C) is another indicator commonly used to evaluate frozen vegetables quality. In spite of its importance for human health (Naidu, 2003), it is generally observed that if this vitamin is well preserved, the other nutrients are also well retained (Lin et al., 1998). In general, a reversible equilibrium

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occurs between ascorbic acid and dehydroascorbic acid (DHAA), which is then irreversibly hydrolysed to 2,3-diketogulonic acid (DKGA) that does not have vitamin C activity. It seems that vitamin C degradation rate is affected by the oxidation–reduction potential of the reaction medium (Serpen and Gökmen, 2007). The oxidizing and reducing agents are naturally present, while some additional species may also occur during thermal treatments. Accordingly, the main mechanisms of ascorbic acid losses during the blanching operation are thermal induced degradation or by leaching (Garrote et al., 1986). During frozen storage the degradation is susceptible to conditions, such as temperature and storage length, being the chemical and enzymatic oxidation the major reasons for AA degradation. The actions of enzymes like AA oxidase, phenolase or peroxidase are also directly or indirectly responsible for vitamin C loss, as referred by Lee and Kader (2000).

As previously referred, enzymes are involved in multiple degradation processes. Consequently, their inactivation is important to reduce colour and some nutrients degradation. Due to its higher thermal resistance, many studies used peroxidase enzyme as an indicator of the thermal treatment applied before freezing vegetables (Anthon and Barret, 2002). It was not found in published works, studies concerning the effect of frozen storage conditions on vegetables' peroxidase kinetics. Normally, researchers discuss the possible reactivation of peroxidase activity after thermal inactivation, under certain frozen storage conditions. However, as Bahçeci et al. (2005) referred, the results are contradictory and peroxidase regeneration normally occurs in purified enzymes.

The objective of this study was to evaluate the effect of blanching and freezing processes on watercress (*N. officinale* R. Br.) colour changes, chlorophylls and ascorbic acid contents, and peroxidase activity, and to evaluate the degradation kinetic parameters for these quality factors during frozen storage at three different temperatures (−7, −15 and −30 °C). This study contributes for the industrial development of a new, nutritive, functional and attractive frozen vegetable product.

Materials and methods

Sample preparation, processing and storage condictions

Fresh watercress (*N. officinale*) was gently supplied by Vitacress, a company that grows watercress in Almancil, Algarve, one day after harvesting. Firstly leaves were sorted, washed and blanched for 20 s at 95 °C, as it was recommended by Cruz et al. (2006), in a thermostatic water bath (±1 °C) with 45 l capacity. During the heat treatment, the temperature of the water was monitored with a digital thermometer (Ellab ctd 87) and a thermocouple (1.2 mm needle dia; constantan-type T). After cooling and drying, watercress was moulded in a parallelepiped form (4.6 cm × 3.3 cm × 1.8 cm) and frozen in an air blast freezer, Armfield FT 36 (Armfield Ltd., Ringwood, England). Samples were frozen at an average air temperature of −40 °C, until the temperature of −25 °C was reached in the sample geometric centre (thermocouple – 1.2 mm needle dia; constantan-type T). Frozen watercress blocks (approximately 90 g) were packed in sealed polyethylene bags and stored in three laboratory chambers (Fitotherm, model S550 BT, Aralab, Portugal) at temperatures of −7, −15 and −30 ± 1 °C, for a total of 13 months, using the same methodology as in Martins and Silva (2003). Samples were randomly taken and analysed raw, after blanching and prior to frozen storage ($t = 0$). Frozen samples were also randomly collected and analysed weekly in the first 5 months, fortnightly in the following trimester and monthly after the 8th month for each storage temperature. All frozen samples were firstly thawed at room temperature during approximately 1 h before analysis.

Colours parameters

The Hunter Lab co-ordinates were measured by a tristimulus colorimeter (model CR 300, Minolta Corporation, Japan) and a CIE standard illuminant C. The colorimeter was calibrated with a standard white tile ($Y = 95.3$, $x = 0.3133$, $y = 0.3197$). Colour was expressed using the L_H, a_H and b_H Hunter scale parameters. Samples were homogenised and placed in a Petri dish for the evaluation. Measurements were taken in triplicates with nine readings.

Combinations of colour parameters may be more effective to evaluate the overall colour changes of processed vegetables than the individual Lab parameters. Therefore, to describe watercress storage colour modifications, the values of $L_H \cdot a_H \cdot b_H$, $-a_H/b_H$, $L_H \cdot a_H/b_H$, and $L_H/a_H \cdot b_H$, as well as the hue parameter (h_H^0), expressed as $h_H^0 = \tan^{-1} b_H/a_H$, that gives the chromatic tonality, were evaluated.

Chlorophylls content determination

A spectrophotometric method adapted by Vernon (1960) was used to quantify chlorophylls a and b. Acetone (Merck) was added with pure water to give a final solution which was 80% in acetone. Watercress samples (2.5 g) were diced and homogenized into a Waring Blendor and 25 ml of acetone solution were added. The samples were homogenized for 3 min. The homogenate was filtered through filter paper Whatman no. 1, with a Büchner funnel under vacuum. The filter cake residues were washed with 80% acetone and the filtrate brought to a final volume of 50 ml with the same solution.

For each sample extract solution an unconverted and a converted sample were required for spectroscopic measurements. The unconverted was prepared by adding 3.0 ml of 80% acetone to a volumetric flask and diluting to 10 ml with the filtered extract. The converted sample was prepared by placing 1.5 ml of saturated oxalic acid in 1.5 ml of 80% acetone in a volumetric flask and diluting to 10 ml with the same filtered extract. Both the control and converted sample were kept in the dark at room temperature for 3 h, after which the absorbances of both samples were determined using 10 mm-path-length glass cells (Amersham Biosciences) in a spectrophotometer (Shimadzu UV 1601, Japan). The solution of 80% acetone and a solution of oxalic acid with 80% acetone with a ratio of 1:1 (v:v) were used as a blank to zero the instrument, respectively, for unconverted and converted samples. All samples were run in three replicates.

Chlorophylls a and b concentrations were calculated using the following equations:

$$\text{Chlorophyll a } (\text{mg}/100\text{g}) = 25.38(\Delta A662) + 3.64(\Delta A645) \quad (1)$$

$$\text{Chlorophyll b } (\text{mg}/100\text{g}) = 30.38(\Delta A645) + 6.58(\Delta A662) \quad (2)$$

The abbreviations $\Delta A645$ and $\Delta A662$ stand for change in absorbance of unconverted and converted sample at 645 and 662 nm, respectively.

Total chlorophyll content was obtained as the sum of chlorophylls a and b contents. The results of chlorophyll concentration are expressed in mg per 100 g of product.

Vitamin C assay

Ascorbic acid determination was carried out by an adaptation of the method developed by Zapata and Dufour (1992). After treatments each sample was homogenized with a Moulinex blender in 20 ml of methanol–ultrapure water (5:95, v/v) during 5 min. Each sample (5 g) was transferred to a 20 ml volumetric flask and 1 ml of isoascorbic acid (IA) standard solution (0.03 g/50 ml) was added. The pH was adjusted with HCl (Merk) to obtain final

values between 2.20 and 2.45. The volume was completed to 20 ml with methanol–ultrapure water (5:95, v/v). The content was centrifuged (Sorval Instruments RC5 C) for 5 min at 10,000 rpm and 4 °C. Afterwards, 3 ml were transferred to another tube with 1 ml of 1,2-phenylenediamine (OPDA) from Sigma (0.03 g/50 ml), daily prepared and maintained in dark. The mixture was then vortexed and placed in dark at room temperature for 40 min. Then the mixture was filtered in a 0.22 μm membrane (Millipore™ – GS Filter), the first milliliter was discarded and 20 μl were injected in the HPLC. The wavelength detector was set to 348 nm, and after elution of DHAA the wavelength was shifted to 262 nm for AA and IA detection. For the mobile phase, 13.61 g of potassium dihydrogen phosphate (Merk) and 3.64 g of cetrimide (Fluka) were added to 2 l of methanol–ultrapure water (5:95, v/v). The eluent was filtered in a 0.45 μm membrane (Macherey-Nagel, Porafil) and degassed in an ultrasonic bath for 15 min. HPLC chromatograms were analysed with the software Gold Neuveau™ Chromatography Data System, Version 1.7©. The results of AA and DHAA were expressed in mg/100 g of sample. Total vitamin C content was obtained as the sum of AA and DHAA contents, quantified by a HPLC technique. All samples were run in six replicates.

Peroxidase activity analysis

Peroxidase activity was determined by a spectrophotometric method adapted from Hemeda and Kleind (1990). The enzyme extract was obtained with 20 g of vegetable sample and 50 ml of distilled water, blended for 2 min in a mixer (Moulinex, France) at room temperature. The homogenate was filtered with membrane filters (Whatman no. 1). The filtrate (100 μl) was mixed to 2.9 ml of substrate solution (prepared daily), (which contained 10 ml of guaiacol (1%, Sigma–Aldrich, G-5502), 10 ml of H_2O_2 (0.3%, Pancreac) and 100 ml of 0.05 M potassium phosphate buffer (pH 6.5)) in a 10 mm-path-length glass cuvettes (Amersham Biosciences). The assay was carried out with a spectrophotometer (Shimadzu UV 1601, Japan). Increase in absorbance at 470 nm was measured with 10 s intervals at 25 °C, during 2 min. Peroxidase activity was defined as a change in 0:001 in absorbance per minute, in the linear region of the curve. Enzyme specific activity was expressed as U/g min. Three replicates per sample, raw, blanched, prior to frozen storage and at each storage time–temperature, were measured.

Data analysis

In general, the studies presented in literature on quality parameters changes during frozen storage of vegetables report zero-order (Eq. (3)) or first-order (Eq. (4)) degradation reaction kinetics,

$$\frac{P}{P_0} = 1 - k_{(T)}t \tag{3}$$

$$\frac{P}{P_0} = e^{-k_{(T)}t} \tag{4}$$

where P is any measured quality factor, the index 0 indicates the initial value, t is the storage time, and k the rate constant at temperature T.

The rate constants at each storage temperature were initially estimated by non-linear regression analysis, fitting the models mentioned above (depending on the quality factor considered). The temperature dependence of the rate constant was found to be mathematically well described by an Arrhenius behaviour:

$$k_{(T)} = k_{\text{ref}} \exp\left[-\frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right] \tag{5}$$

where k_{ref} is the rate constant at a reference temperature, T_{ref}, Ea the activation energy, and R the universal gas constant. The reference temperature used was the middle value of the range considered (i.e. $T_{\text{ref}} = -15$ °C). The temperature effect can be directly included in quality factors prediction, by substitution of Eq. (5) into the kinetic model. Therefore, the factor k_{ref} and the Ea were estimated directly from experimental data in a *one-step* (quality factor *versus* time, at all temperatures), by performing a global non-linear regression analysis, merging the Arrhenius equation and the kinetic models considered (Arabshahi and Lund, 1985; Lund, 1983).

Parameterś precision was evaluated by confidence intervals at 95%. The quality of the regression was assessed by the coefficient of determination (R^2), and randomness and normality of residuals (Hill and Grieger-Block, 1980), thus allowing best model selection.

Statistica version 6.0 software (Stata Corporation, 1999) was used for all regression analysis procedures (using least squares estimation and Levenverg–Marquart method for minimising sum of squares of the deviations between experimental values and the ones predicted by the mathematical model).

An analysis of variance (one-way ANOVA with replication) was performed to assess the blanching and frozen operation effects on quality parameters. An analysis of variance (factorial ANOVA with replication) was also performed to evaluated storage time–temperature effects on quality parameters.

Results and discussion

Changes in colour parameters

Processed watercress colour parameters presented significant differences compared with raw product (Table 1). Watercress became lighter and more green (a_H parameter is more negative) and yellow (b_H parameter increased). The majors changes were due to the blanching treatment, where the L_H, a_H and b_H parameters changed, respectively, 11%, 69% and 31%.

Table 1
Watercress quality parameters evaluated on fresh, thermally treated and after freezing (mean values ± standard deviation).[a]

Parameters		Fresh	Thermally treated	Frozen
Peroxidase activity (U/g min × 10^6)		460.44 ± 83.71[a]	68.67 ± 14.55[b]	0.32 ± 0.036[c]
Colour	L_H	22.17 ± 0.08[a]	24.63 ± 0.39[b]	25.48 ± 0.24[c]
	a_H	−5.17 ± 0.24[a]	−8.72 ± 0.26[b]	−9.54 ± 0.34[c]
	b_H	6.98 ± 0.21[a]	9.14 ± 0.26[b]	9.78 ± 0.31[c]
Chlorophylls (mg/100 g)	Chlorophyll a	117.59 ± 12.56[a]	75.26 ± 6.23[b]	65.64 ± 3.30[b]
	Chlorophyll b	22.61 ± 3.60[a]	16.73 ± 3.15[b]	15.64 ± 12.71[b]
	Total chlorophylls	140.21 ± 16.23[a]	92.34 ± 17.86[b]	81.28 ± 15.71[b]
Vitamin C (mg/100 g)	Ascorbic acid (AA)	36.75 ± 2.11[a]	40.57 ± 2.07[a]	37.59 ± 3.55[a]
	Dehydroascorbic acid (DHAA)	28.08 ± 4.47[a]	16.72 ± 0.28[b]	7.21 ± 0.68[c]
	Total ascorbic acid	64.83 ± 7.84[a]	57.30 ± 2.27[a]	44.80 ± 4.20[b]

[a] Values in the same row followed by the same letter are not significantly different (LSD, $P = 0.05$).

Watercress colour L_H values did not change significantly ($P \geqslant 0.05$) during 400 days of frozen storage, describing a random pattern behaviour around an average value (±standard deviation) of 23.70 ± 0.92. On the other hand, a_H values at the storage temperature of −7 °C were significantly different from the values obtained with the other two temperatures evaluated. At this temperature a_H values increase from −9.54 ± 0.34 until a final value of −4.44 ± 0.30, reflecting a change in green colour. At −15 and −30 °C the a_H values were almost stable. Moreover, at the higher storage temperature (−7 °C) watercress became significantly ($P \leqslant 0.05$) more yellow, more 25% in relation to samples at $t = 0$ (prior to frozen storage) ($b_H = 9.78 \pm 0.31$) (data not shown).

Fig. 1 presents hue (h_H^0) colour values of frozen watercress at three different storage temperatures. At the higher temperature of −7 °C, a significant reduction in hue angle is observed, which corresponds to a decrease in the greenness intensity and an increase in yellowness. In relation to the other colour combinations of parameters evaluated, $L_H \cdot a_H \cdot b_H$, $-a_H/b_H$, $L_H \cdot a_H/b_H$, and $L_H/a_H \cdot b_H$, the behaviour was similar to the hue parameter (data not shown). All parameters combinations suffer significant changes, at a temperature of −7 °C, during storage.

A zero-order kinetics model (Eq. (3)), with Arrhenius temperature dependence (Eq. (5)), fitted well the experimental data for hue and all these combinations of parameters. The rate constant at reference temperature and activation energy are presented in Table 2. The correlation coefficient (R^2) values were greater than 0.80, while the standard errors (SE) were less than 10^{-5} for the entire range. Therefore, all the colour parameters combinations ap-

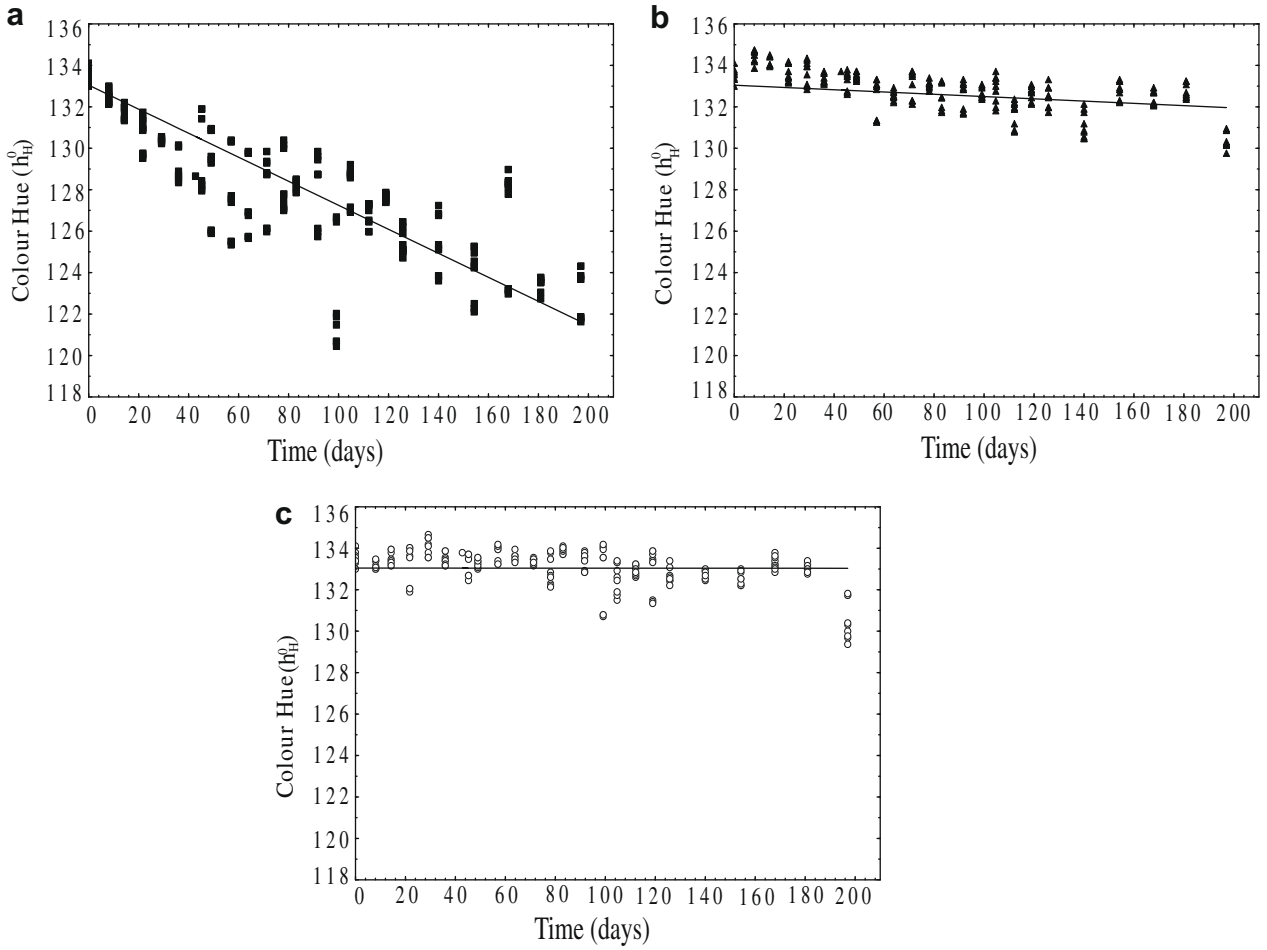

Fig. 1. Watercress Hunter hue (h_H^0) during frozen storage (■, experimental data at −7 °C; ▲, experimental data at −15 °C; ○, experimental data at −30 °C). The lines represent model fits (Eqs. (3) and (5) one-step) to experimental data.

Table 2
Kinetic parameters, and corresponding confidence intervals at 95%, of watercress peroxidase inactivation, colour and ascorbic acid degradation, due to frozen storage.

Quality factor (kinetic model)		Kinetic parameters		
		P_0	$k_{-15°C}$ (days^{-1}) $* 10^{-3}$	E_a (kJ mol^{-1})
Colour (Arrhenius zero-order; Eqs. (3) and (5))	h_H^0	133.04 ± 0.15	5.48 ± 2.22	168.65 ± 28.18
	$L_H * (-a_H) * b_H$	1880.71 ± 31.35	556.85 ± 278.12	133.63 ± 34.57
	$L_H/(-a_H) * b_H$	27.11 ± 0.16	2.04 ± 1.45	191.74 ± 49.66
	$L_H * (-a_H)/b_H$	22.57 ± 0.16	2.93 ± 1.39	158.88 ± 33.06
	$-a_H/b_H$	0.91 ± 0.0049	0.094 ± 0.04	164.66 ± 32.25
Ascorbic acid (AA) (Arrhenius first-order; Eqs. (4) and (5))		32.62 ± 1.34 mg/100 g	4.32 ± 0.45	24.73 ± 4.52
Peroxidase inactivation (Arrhenius first-order; Eqs. (4) and (5))		1.92×10^5 ± 0.14 (U/g.min)	2.58 ± 0.58	17.34 ± 7.78

pear to be adequate to describe watercress colour degradation during frozen storage. The high activation energies of all colour combination parameters (values between 133.63 and 191.74 kJ mol^{-1}) are related to watercress temperature sensitivity, especially at higher frozen storage conditions.

In a study published by Martins and Silva (2002), on the kinetics of colour degradation in frozen green beans, similar behaviour was observed, but the change in the rate of degradation was modelled as fractional conversion model.

Losses in chlorophylls contents

Fresh watercress chlorophylls a and b contents (±standard deviation) were found to be 117.59 ± 12.56 and 22.61 ± 3.60 mg/100 g, respectively. The content of chlorophylls quoted by Lisiewska et al. (2004) in dill was in the same range obtained in the present work. The thermal treatment induced a significant ($P \leqslant 0.05$) decrease in the level of the total chlorophylls (34%). Moreover, chlorophyll a decreased faster than chlorophyll b, respectively, 36% and 26%, in agreement with the results obtained by Gökmen et al. (2005) in peas. On the other hand, freezing did not induce significant chlorophylls changes ($P \geqslant 0.05$). The effect of storage time and temperature on watercress chlorophylls a and b contents is presented in the Fig. 2. It can be observed that at a temperature of −7 °C the chlorophyll a values have a slight decrease during the 400th storage days, from an initial value of 65.64 ± 3.30 till 46.85 ± 2.54 mg/100 g of product. For the other tested temperatures (−15 and −30 °C), the chlorophyll a values remained approximately constant (c.a. 67 mg/100 g average value). Comparing the results with those reported by Lisiewska et al. (2004) for other blanched vegetables leaves, lower temperatures applied during the frozen storage preserved better the chlorophylls content, due to a decrease in the rate of chemical degradation reactions.

Previous studies have found relationship between chlorophyll content and physical colour measurements on different frozen vegetables (Koca et al., 2006). For frozen watercress storage at three different temperatures, however, none of the chlorophyll components gave significant ($P \geqslant 0.05$) correlation with any determined colour parameters or combination. Such discrepancies, between colour and chlorophyll reaction rates, are attributed in most cases to chlorophyll method accuracy (Venning et al., 1989; Martins and Silva, 2002).

Losses in vitamin C content

Fresh watercress presents higher total ascorbic acid content (see Table 1) than most commonly consumed vegetables (peas 31–26 mg/100 g, green beans 25–10 mg/100 g, carrots 4 mg/100 g, spinach 31–22 mg/100 g and tomatoes 14 mg/100 g) with the exception of broccoli (97–77 mg/100 g) (Giannakourou and Taoukis, 2003; Lee and Kader, 2000). This result demonstrates that watercress is a good vitamin C source and an important vegetable for the human diet. Blanching did not cause a significant decrease in total ascorbic acid (AA + DHAA) content (only 12% was lost),

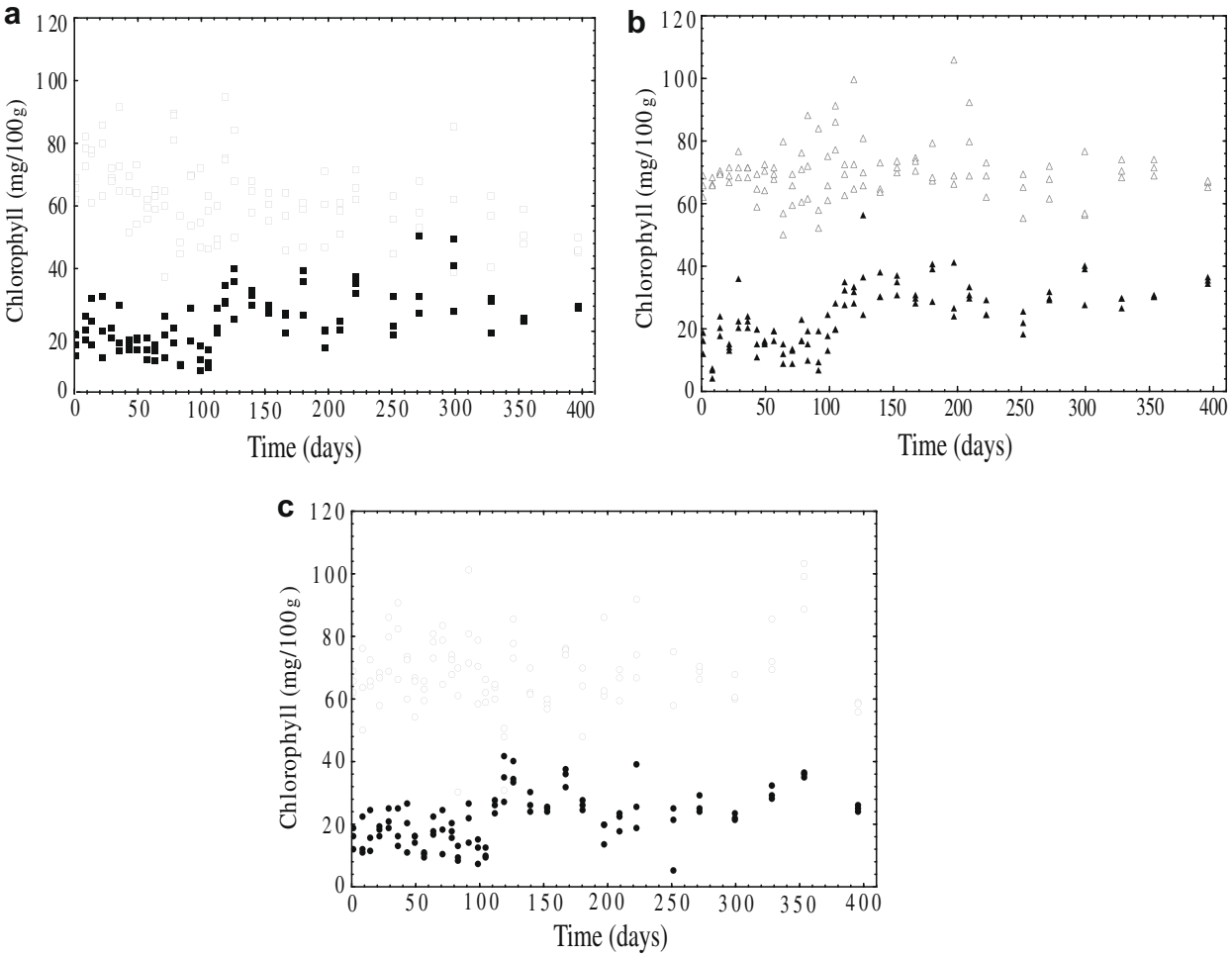

Fig. 2. Effect of frozen storage on watercress chlorophylls a (open symbols) and b (black symbols) content. Values at (a) −7 (□), (b) −15 (△) and (c) −30 °C (○).

suggesting that processing conditions were adequate to minimise the thermal impact on quality attributes. Other studies reported, for six species of leafy vegetables, decreases in the order of 52–81% (Ajayi et al., 1980). However, the blanching and freezing operation of watercress led to a statistically significant ($P \geqslant 0.05$) decrease in the content of the DHAA component. As no equilibrium was detected between AA and DHAA fraction, this situation suggests that degradation of DHAA occurs due to hydrolysed reaction.

The ascorbic acid (AA) content (±standard deviation) in frozen watercress, evaluated prior to frozen storage ($t = 0$), was 37.59 ± 3.55 mg/100 g. After 400 days of isothermal storage, AA content gradually decreased 95%, 93% and 76%, respectively, at the temperatures of −7, −15 and −30 °C (Fig. 3). These results are in agreement with other reported by Bahçeci et al. (2005) for other vegetables species. Experimental results for watercress AA losses were satisfactorily described by an Arrhenius first-order kinetic model ($R^2 = 0.79$). Fig. 3 includes also the model fit obtained by a one-step regression analysis. The quality of the model fit was assessed by analyses of the residuals (i.e. normality and randomness were confirmed). The kinetic parameters are given in Table 2. In comparison with the activation energy values reported by Giannakourou and Taoukis (2003) for AA content in different frozen vegetables (range of 98–112 kJ mol^{-1}), and Martins and Silva (2003) in frozen green beans (42 kJ mol^{-1}), the value for watercress is higher. Consequently, frozen watercress ascorbic acid appears to be less sensitive to changes in storage temperature.

DHAA contents of fresh, blanched and prior to frozen storage watercress samples are presented in Table 1. Blanching and freezing decreased significantly ($P \geqslant 0.05$) DHAA content, approximately 40% and 74%, respectively. During watercress storage no significant loss was observed (Fig. 3). The average value of the analysed component (c.a. 6 mg/100 g) does not depend upon the storage period of time or temperature. Martins and Silva (2003) and Bahçeci et al. (2005) in frozen green beans, observed that DHAA contents were stable or tended to decrease with storage time, respectively. Serpen et al. (2007), in frozen peas observed, that DHAA content was better retaining during storage if samples were blanched.

POD activity measurements

The POD activity (±standard deviation) in fresh, blanched and frozen before storage watercress leaves is presented in Table 1. The applied thermal treatment inactivated approximately 85% of POD activity. The complete enzymes inactivation is not a requirement of the blanching process, as it was referred by Günes and Bayindirh (1993). The objective of the blanching should be the stabilization of the frozen product during storage. The applied binomial time–temperature appears to be adequate compared with other studies in vegetables leafs, namely spinach and Swiss chard, with blanching times ranging from 15 to 30 s at 95 °C (Agüero et al., 2005).

After freezing, POD presented only a residual activity of about 0.07%. The observed activity reduction due to freezing may be explained by events such as enzyme aggregation and/or conformational changes (Manzocco et al., 1999; Lisiewska and Kmiecik, 2000).

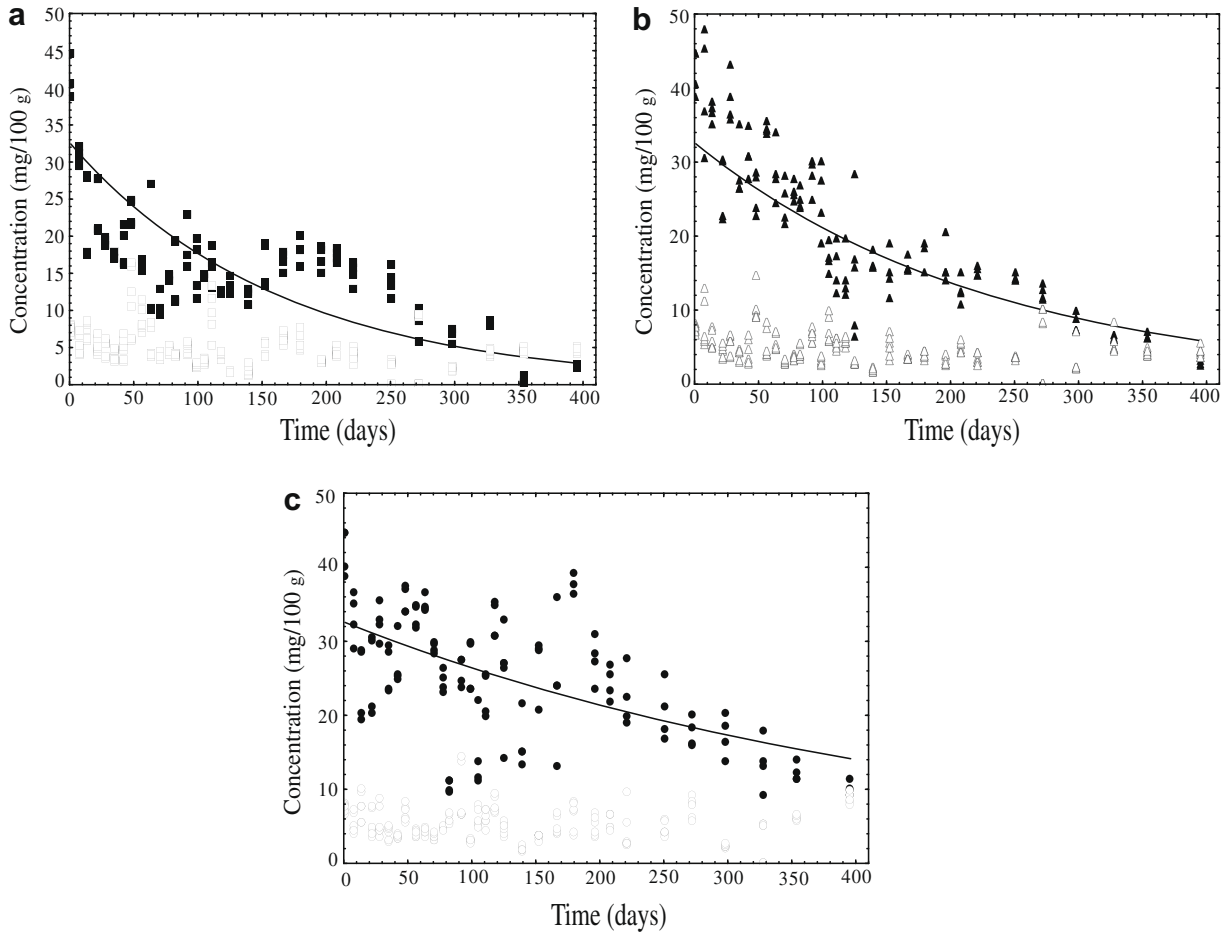

Fig. 3. Effect of frozen storage on watercress ascorbic acid (AA) (black symbols) and dehydroascorbic acid (DHAA) (open symbols) content. Values at (a) −7 (□), (b) −15 (△) and (c) −30 °C (○). The lines represent model fits (Eqs. (4) and (5) one-step) to experimental data.

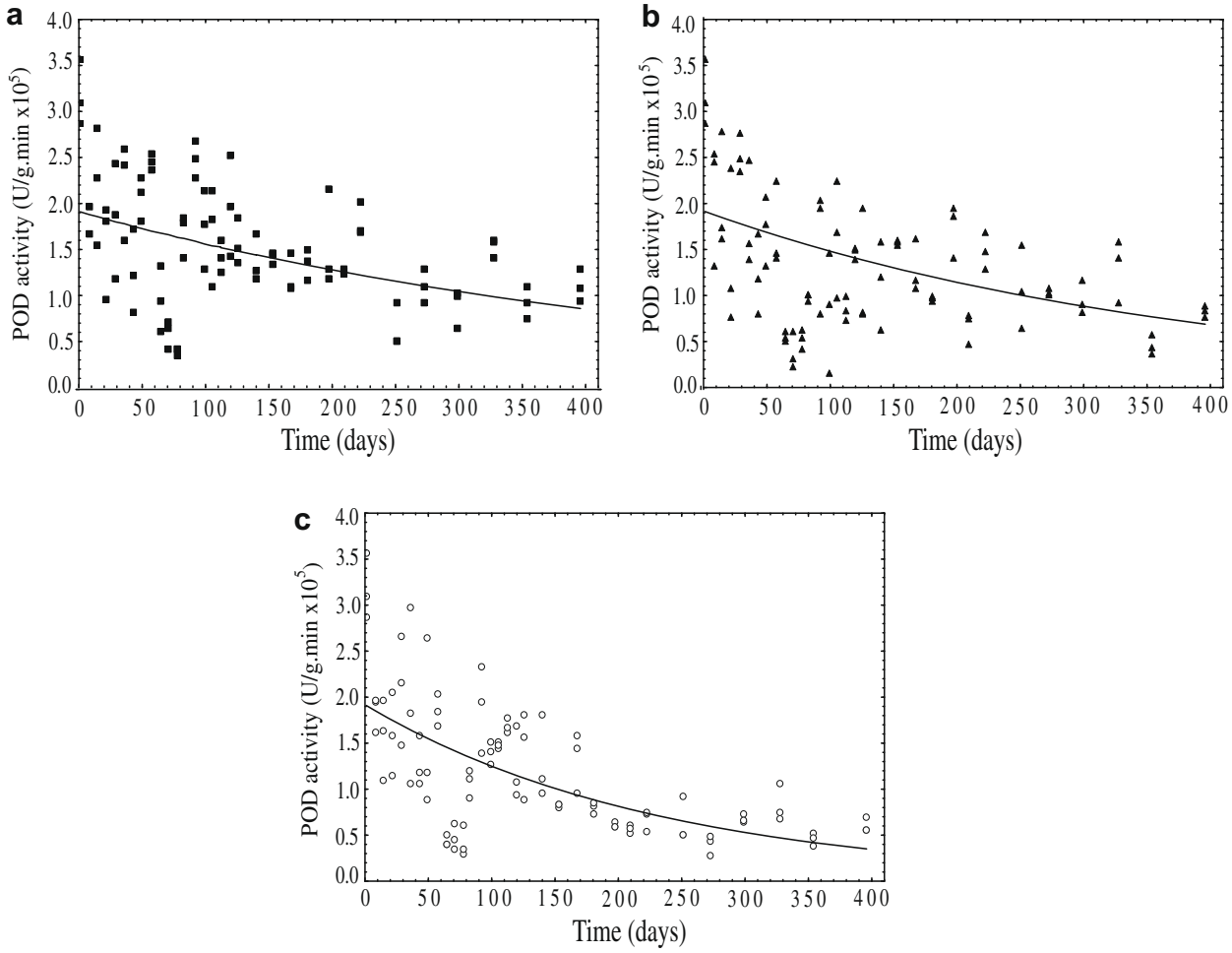

Fig. 4. Watercress peroxidase residual activity (POD) during frozen storage (■, experimental data at −7 °C; ▲, experimental data at −15 °C; ○, experimental data at −30 °C). The lines represent model fits (Eqs. (4) and (5) one-step) to experimental data.

The POD residual activity decreased with time of storage, following an identical pattern for the three temperatures (Fig. 4). The decrease (comparing with the first storage day) was greater at lower temperatures, 79.5% at −30 °C, 74.7% at −15 °C and 71.2% at −7 °C. Similarly results were noted by Steinbuch et al. (1979) in Brussels sprouts. This activity reduction could be attributed to higher enzyme denaturation, as was referred by Gökmen et al. (2005). In agreement with the study reported by Bahçeci et al. (2005), no regeneration of POD activity was observed during the period of storage.

Experimental results on frozen stored watercress POD residual activity were satisfactorily described by an Arrhenius first-order kinetic model. Fig. 4 includes the model fit obtained by one-step regression analysis. 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 0.72. Estimated activation energy, rate constant at the reference temperature of −15 °C, and corresponding 95% confidence intervals are included in Table 2.

Conclusions

The applied blanching treatment (95 °C during 20 s) to watercress (*N. officinale* R. Br.) reduced 85% its POD activity. This reduction seems to be sufficient to promote enzyme stability during frozen storage. However, the thermal treatment induced significant change on colour values, chlorophylls and vitamin C contents. Also, colour parameters and POD residual activity were significantly affected by the freezing operation.

Peroxidase and ascorbic acid (AA) contents in frozen watercress followed the first-order degradation kinetics. The other quality factors analysed (colour, different parameters) were well described by a zero-order model. The Arrhenius model described the temperature dependence of the reaction rate constant for all the considered factors. No considerable changes on chlorophylls and dehydroascorbic acid (DHAA) contents were observed over the whole temperature and time ranges of frozen storage studies.

Finally, considered only the two main temperature sensitive indices, colour and AA, and if, for example 50% of AA loss was taken as a limit, shelf life of about 6 month at temperature recommended (−18 °C) would be expected. Therefore, despite the degradation of some watercress quality parameters during the freezing process and frozen storage, the above results clearly show that freezing can act has a suitable process in watercress preservation.

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