Degradation kinetics of colour, vitamin C and drip loss in frozen broccoli (*Brassica oleracea* L. ssp. *Italica*) during storage at isothermal and non-isothermal conditions

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A B S T R A C T

Studies were undertaken on colour CIE $L^*a^*b^*$ values, vitamin C (ascorbic acid) and drip loss alterations of frozen broccoli (*Brassica oleracea* L. ssp. *Italica*) stored at isothermal (-7 , -15 , and -25 °C) and non-isothermal (accelerated life testing with step-stress methodology; temperature range from -30 to -5 °C) conditions. The storage temperatures were selected according to conditions that occur in the cold chain.

Frozen storage at all regimes had significant impact on all quality parameters analysed. Significant alterations in broccoli green colour, vitamin C content and drip loss were observed.

Experimental data of h^* colour degradation and drip loss (%) at isothermal conditions and a^*/a_0^* at non-isothermal conditions could be modelled by zero order kinetics. A first order kinetic model was adequate for the remaining quality factors and temperature regimes. The effect of storage temperature on kinetic parameters was successfully described by the Arrhenius equation.

Cinétique de détérioration de la couleur, de la teneur en vitamine C et exsudats du brocoli (*Brassica oleracea* L. ssp. *Italica*) pendant l'entreposage sous des conditions isothermes et non isothermes

Mots clés : brocoli ; surgélation ; entreposage ; qualité ; durée de conservation ; modélisation

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1. Introduction

Broccoli (*Brassica oleracea* L. ssp. *Italica*) is a cruciferous vegetable rich in health-promoting compounds, such as fibre, vitamin C, glucosinolate and flavonoid, and selenium. These compounds have gained attention because of their role in the prevention of cancer and cardiovascular diseases (Finley et al., 2000; Keck and Finley, 2004). After harvest, broccoli is a highly perishable vegetable that rapidly (in few days) loses its bright green colour. Lowering the temperature (till 0–4 °C) and increasing the relative humidity (till 95–100%) extend the vegetable shelf-life for one to two weeks (Jones et al., 2006).

Freezing of vegetables is one of the most common processes for quality preservation, because at those low temperatures deteriorative reactions are reduced to minimal rates and microorganism's growth is restricted. However, the quality of frozen vegetable is highly dependent on the characteristics of the raw food (Simandjuntak et al., 1996), environmental conditions to which the vegetable is exposed after being subjected to the freezing process (Alvarez and Canet, 2000; Sahari et al., 2004), pre-freezing operations such as blanching (Jaworska et al., 2008; Olivera et al., 2008), and the freezing process itself.

Inevitably, the freezing process and the frozen storage have negative impacts on food quality. The main physical changes that occur during frozen storage are moisture migration and ice recrystallization (Van Buggenhout et al., 2006b). Both phenomena are related to the stability of products' frozen water, which affects the vegetable texture and loss of nutrients and weight if thawing drip loss takes place (Pukszta and Palich, 2007). Moreover, chemical changes in frozen vegetables are related with onset of off-odour and off-flavours (especially if vegetables were not blanched; Nielsen et al., 2003), pigment and colour degradation (Lisiewska et al., 2004) and chemical oxidation (e.g. of vitamin C; Serpen et al., 2007).

The (EU Directive 89/108) recommends that storage temperature must be stable and maintained below –18 °C for a storage period between 6 and 24 months. However, quality of frozen food is highly dependent on storage temperature, with lower temperatures leading to longer shelf-life. Quality deterioration is emphasised by storage temperatures variations that often occur in the frozen chain, and that are consequences of temporary breakdown of facilities or equipment, improper response of temperature controlling devices, excessive loads during freezing or unacceptable handling (Taoukis et al., 1991). The knowledge of the exact influence of storage temperature for each frozen vegetable would help to determine adequate conditions to enhance quality and determine its shelf-life.

In general, microbiological criteria are of primary importance to established food products' shelf-life, specially for the highly perishable ones (Corbo et al., 2006). However, the frozen vegetables shelf-life is normally established by sensory evaluation (IIR, 1986). Since results are based on subjective decisions, Giannakourou and Taoukis (2003), Reid et al. (2003) and Martins and Silva (2004a) proposed the use of objective measurements, such as colour and texture, and content of nutritional compounds, e.g. vitamin C, that can be considered an important back-up to sensory methods. Even so, no well-

defined criteria have been until now established and it is important to identify which factor is of utmost importance in the frozen vegetable shelf-life definition.

Normally, studies to assess the impact of frozen storage on products' stability require sampling throughout long-term periods. For this reason, different methodologies have been developed to accelerate shelf-life testing (ALT), which, in general terms, refers to any method that is capable of evaluating product stability, based on data that are obtained in a significantly shorter period than the actual shelf-life of the product. Authors like Martins and Silva (2004b), Hough et al. (2006), Corradini and Peleg (2007) and Martins et al. (2008) used accelerate shelf-life testing and discussed different modelling approaches and how they can be used in shelf-life predictions. Several of these models were applied to evaluate/predict quality and shelf-life of a variety of food products during frozen storage: potatoes (Alvarez and Canet, 1998), green beans (Martins et al., 2005), watercress (Cruz et al., 2009), dough and bread (Phimolsiripol et al., 2008), meat (Berry and Leddy, 1989) and fish and shrimp (Koutsoumanis et al., 2002; Tsironi et al., 2009).

Therefore, the objectives of this work were to study the effect of the freezing process (with a pre-blanching step) and frozen storage on some quality factors of broccoli. Kinetic models were fitted to data obtained under isothermal and non-isothermal frozen storage, using accelerated life testing methodology. Determination of frozen broccoli shelf-life was also a target.

2. Materials and methods

2.1. Raw material

Fresh broccoli (*Brassica oleracea* L. ssp. *Italica* cv. Marathon) was obtained directly from cultivated area in Torres Vedras, Portugal. Leaves were removed and broccoli inflorescences were divided into florets of about 5 cm diameter, cut 2 cm below the lowest ramification.

2.2. Pre-blanching and freezing process

For blanching treatments, samples were immersed in a thermostatic water bath (± 1 °C) of 50 L capacity. The blanching conditions (i.e. 70 °C for 6.5 min) were the ones optimized by Gonçalves et al. (2009b). The temperatures of the water and samples were monitored throughout the thermal treatment, using 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 blanching, samples were cooled into an ice water bath for 2 min; excess of moisture was removed before any further analysis and/or process.

Blanched samples were frozen in a vertical forced air freezer (Refriger, Electro-Refrigeração, Portugal) at –40 °C (average value), until the temperature of the centre of samples reached –35 °C. Temperature was recorded with thermocouples (type T thin thermocouple, 1.2 mm diameter, embedded in a stainless steel hypodermic needle, Ellab, Denmark, with an accuracy of ± 2 °C). Frozen broccoli (~500 g each) was

immediately packed into polyethylene bags (22 × 35 cm) and sealed.

2.3. Frozen storage regimes

After freezing, two frozen storage regimes (I and II) were considered.

2.3.1. Regime I

Corresponds to isothermal frozen storage conditions at −7, −15 and −25° (±1 °C). Frozen samples were distributed into three laboratory freezers (Fitotherm, S550 BT) at the constant temperatures established. Samples were removed at heuristic time intervals (i.e. sampling tended to be equally spaced in the time scale), over a period of 121 days.

2.3.2. Regime II

Corresponds to non-isothermal frozen storage conditions, considering accelerated life testing (ALT) with step-stress methodology (i.e. consecutive increases in temperature, by step levels). Samples were stored in sequence at −30 °C (for 21 days), −20 °C (for 16 days), −10 °C (for 11 days) and −5 °C (for 9 days), totalling 57 days. Samples were removed at heuristic time intervals (i.e. sampling tended to be equally spaced in the time scale).

Air and product temperatures were monitored using type T thermocouples connected to an acquisition data system with software LabView 8.2 (National Instruments) and an OMB-DAQ-56 multi function data acquisition module (Omega Engineering, Stamford, CT).

Before any further analysis, samples were thawed at 4 °C for 15 h in a refrigerator with air temperature control. This period of time was enough to reach the same temperature value of all samples (4 °C), regardless the different temperatures used in the frozen regimes.

2.4. Quality analysis

2.4.1. Peroxidase enzyme

Peroxidase (POD) activity was measured as the change in absorbance at 420 nm using guaiacol and H₂O₂ as substrates, based on a modified method of Bifani et al. (2002) as described in Gonçalves et al. (2009b). Raw, blanched and frozen (prior to the frozen storage and at the last day of frozen storage) samples were analysed in triplicate.

2.4.2. Colour evaluation

For colour determinations, 30 g of broccoli were triturated and analysed 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. 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 colour behaviour of broccoli during frozen storage, the normalized (a*/a₀*) value and the hue angle, h* (0–360°) (obtained by tan^{−1} b*/a*, expressing the characteristic/dominant colour), were used. Data was obtained from 20 measurements on each sample.

2.4.3. Vitamin C

In order to determine vitamin C (ascorbic acid) content, a modified titrimetric AOAC method (1984) was used. 200 g of each sample were blended with an equal weight of metaphosphoric acid (6%) using a Waring commercial blender (Dynamics Corporation, CT, USA). 20 g of the macerate was added to 100 ml of metaphosphoric acid (3%) and filtered through Whatman n° 42 filter paper. A filtered sample of 10 ml was titrated with 2,6-dichlorophenolindophenol until persistent pink colour was observed. Three independent extractions were performed per sample and results were expressed as mg ascorbic acid per 100 g of broccoli samples.

2.4.4. Drip loss

Frozen samples were laid over a weighted absorbent paper and let to thaw at 4 °C (Agnelli and Mascheroni, 2002). Drip loss (DL) was then evaluated by periodically weighting the absorbent paper until a constant value was reached:

$$DL(\%) = \frac{(W_t - W_0)}{W_s} \times 100 \quad (1)$$

where W₀ is the weight of the dry absorbent paper (g), W_t the weight of the wet absorbent paper at time t (g) and W_s the weight of the frozen sample (g). A Mettler AT 100 analytical balance with metering precision of 0.00001 g was used. Measurements were made in triplicate per sample.

2.5. Modelling procedures

2.5.1. The models

Under isothermal frozen conditions, kinetics of quality changes may be described by a zero or first order kinetic models, Eqs. (2) and (3) respectively, depending on the parameter considered.

$$C = C_0 - k_{0(T)}t \quad (2)$$

$$\frac{C}{C_0} = e^{-k_{1(T)}t} \quad (3)$$

where C is the measured quality factor, t is the storage time and k the reaction rate (the subscripts 0 and 1 correspond to zero and first order k values, respectively).

The dependence of the reaction rate on temperature is commonly described by an Arrhenius behaviour:

$$k = k_{ref} \exp \left[-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \quad (4)$$

where k_{ref} and E_a are model parameters, the kinetic rate at a finite reference temperature (T_{ref}) and the activation energy, respectively; R is the universal gas constant.

A global model that includes the temperature effect can be obtained by merging Eqs. (2) and (4) or (3) and (4), thus giving:

$$C = C_0 - \left(k_{ref} \exp \left(-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right) t \right) \quad (5)$$

$$C = C_0 \exp \left(-k_{\text{ref}} \exp \left(-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \right) t \right) \quad (6)$$

Under non-isothermal conditions, and if the variation of temperature with time is known ($T(t)$), the previous equations integrate to:

$$C = C_0 - \left[k_{\text{ref}} \int_0^t \exp \left[-\frac{E_a}{R} \left(\frac{1}{T(t)} - \frac{1}{T_{\text{ref}}} \right) \right] dt \right] \quad (7)$$

$$C = C_0 \exp \left[-k_{\text{ref}} \int_0^t \exp \left[-\frac{E_a}{R} \left(\frac{1}{T(t)} - \frac{1}{T_{\text{ref}}} \right) \right] dt \right] \quad (8)$$

2.5.2. Data analysis

For isothermal conditions, Eqs. (5) and (6) were fitted to experimental data using Statistica[®] 8.0 (2007), performing a global non-linear regression analysis (quality factor data versus time, at all temperatures) (Lund, 1983; Arabshahi and Lund, 1985).

For non-isothermal conditions, Eq. (7) or (8) were fitted to experimental data. The regression analysis procedures and calculations were performed in programs specially written in FORTRAN 77 language (Fortran 5.1, Microsoft Corporation[®], 1990). The simplex algorithm was used to minimise the sum of the squares of the residuals (Nelder and Mead, 1965).

The reference temperature assumed was -15°C in all cases (i.e. the average value of the experimental range considered) aiming at improving parameter estimation.

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 (Weemaes et al., 1999), thus testing model adequacy.

An analysis of variance (factorial ANOVA with replication) was also performed to evaluate storage time-temperature effects on quality parameters. Pearson correlation coefficients were also calculated to assess correlations between quality measurements.

3. Results and discussion

Pre-blanching inactivated approximately 90% of initial POD activity ($712.1 \pm 57.3 \text{ Ug}^{-1}$). No peroxidase regeneration was detected during the storage period at different temperature regimes. Therefore, enzymatic activity should not be considered responsible for changes that occurred throughout storage, such as colour or vitamin C (ascorbic acid).

3.1. Colour changes

Broccoli colour is the characteristic with most influence on consumer choice. Chlorophyll pigments are responsible for the green colour of many vegetables, namely broccoli. Colour alterations during frozen storage are attributed to the fade of the vivid green colour of chlorophyll to an olive brown, characteristic of pheophytin (Martins and Silva, 2002). The blanched/frozen broccoli (the reference sample before frozen storage) exhibited a green colour, corresponding to the following average values of the colour coordinates: $L^* = 22.04 \pm 1.91$, $a^* = -17.41 \pm 0.75$, $b^* = 15.97 \pm 1.13$ and $h^* = 137.95 \pm 3.91$.

3.1.1. Isothermal storage conditions

During the isothermal frozen storage at the lowest temperatures (-25 and -15°C), the L^* value showed a random pattern behaviour around its initial value. However, at -7°C , broccoli became lighter and L^* value increased 53% until the last day of storage (data not shown). The normalized a^* (a^*/a_0^*) and h^* values (Fig. 1a and b, respectively) decreased significantly ($P \leq 0.05$) with the increase of time and temperature of storage, reflecting a change in broccoli green colour. An identical behaviour was also reported by Martins and Silva (2002) and Gonçalves et al. (2009a) in green beans and watercress, respectively. No significantly changes were observed in b^* values for the three storage temperatures. However, at -7°C a slight alteration was observed; broccoli became more yellow (13% of variation; data not shown).

Experimental results of broccoli a^*/a_0^* and h^* colour parameter degradation were satisfactorily described by an Arrhenius first and zero order kinetic model, respectively. Fig. 1 (a and b) also includes the model fit obtained by a one-step regression analysis (i.e. global fit of data at all temperatures). In all cases normality and randomness of residuals were verified, thus proving model adequacy. In spite of the high variability of broccoli colour data, the coefficient of determination, R^2 , was satisfactorily high (0.85 for both situations). The kinetic parameters are given in Table 1. The activation energies found by Martins and Silva (2002) for green beans and Gonçalves et al. (2009a) for watercress were substantially higher (103 and 174 kJ mol^{-1} for a_H and h^* colour parameters, respectively).

3.1.2. Non-isothermal storage conditions

At the end of storage under non-isothermal conditions (57 days), broccoli colour parameters a^*/a_0^* and h^* decreased 30 and 5% respectively (Fig. 1c and d). The degree of greenness was more affected than the degree of tonality. These alterations were lower than the ones observed under isothermal storage at -7°C for the same period of time (a^*/a_0^* degraded 47% and h^* 13%), but were higher than the ones obtained at -25°C (a^*/a_0^* degraded 17%, and h^* 3%).

Under non-isothermal conditions, first and zero-order kinetic models Eqs. (7) and (8) were adequate in describing a^*/a_0^* and h^* changes, respectively (model fits are included in Fig. 1 c and d; parameters estimates are in Table 1). The coefficients of determination were 0.40 and 0.44, respectively for a^*/a_0^* and h^* , revealing a great dispersion of experimental data. Nevertheless, analyses proved residuals randomness and normality, and consequently there is statistically supported evidence that models were adequate in data fitting.

The kinetics of broccoli colour alterations under non-isothermal conditions presented considerably lower activation energy values, when compared to isothermal conditions. The values were also lower than the ones reported by Martins et al. (2005) for green beans colour parameters (e.g. for a_H parameter, $111.9 \text{ kJ mol}^{-1}$).

3.2. Vitamin C losses

Vitamin C is a water soluble vitamin that is widely considered as an appropriate marker for monitoring quality changes

Fig. 1 – Broccoli colour degradation during frozen storage: under isothermal conditions (a) a^*/a_0^* and (b) hue (h^*) parameters; under non-isothermal conditions (c) a^*/a_0^* and (d) hue (h^*) parameters (dotted lines indicate temperature history of regime II).

during processing, storage and at the end point of the frozen chain. This is a consequence of its concentration change due to irreversible oxidation mechanisms that are enhanced by temperature abuses (Serpen et al., 2007).

Even after the blanching and freezing operations, broccoli (before frozen storage; the reference sample) presented a vitamin C (ascorbic acid) content of 36.07 ± 0.98 mg/100 g, which is higher than the values reported for other green fresh

Table 1 – Kinetic parameters, and corresponding confidence intervals at 95%, of broccoli colour parameters, vitamin C (ascorbic acid) content and drip loss due to isothermal and non-isothermal frozen storage.

Frozen storage	Quality factor	Model	Kinetic parameters			
			C_0	$k_{-15^\circ\text{C}} \times 10^3 \text{ (days}^{-1}\text{)}$	E_a (kJ mol ⁻¹)	
Isothermal	Colour	a^*/a_0^*	0.87 ± 0.012	4.35 ± 0.38	53.58 ± 5.58	
		h^*	134.69 ± 0.52	0.90 ± 0.091	58.96 ± 6.78	
	Vitamin C (ascorbic acid)	(mg/100g)	32.91 ± 0.70	6.80 ± 0.69	60.24 ± 7.20	
		(%)	11.26 ± 0.24	42.66 ± 4.73	42.31 ± 6.89	
Non-isothermal	Colour	a^*/a_0^*	1.03	6.91	0.017	
		h^*	137.23	0.81	0.11	
	Vitamin C (ascorbic acid)	(mg/100g)	34.73	7.55	0.0039	
		(%)	12.15	366.80	0.0047	

The subscripts 0 and 1 correspond to zero and first order kinetics, respectively.

vegetables (green beans 25–10 mg/100 g; spinach 31–22 mg/100 g; peas 26.5 mg/100 g [Serpen et al., 2007](#); [Martins and Silva, 2002](#)). Similar (34 mg/100 g) and higher (60 mg/100 g) frozen broccoli vitamin C contents were reported by [Sikora et al. \(2008\)](#) and [Gębczyński and Lisiewska \(2006\)](#), respectively.

3.2.1. Isothermal storage conditions

The effect of frozen storage at different temperatures on vitamin C degradation is in [Fig. 2a](#). After 121 days of isothermal storage, the vitamin C content significantly decreased c.a. 80%, 60% and 29%, respectively at -7 , -15 and -25 °C. The major loss of vitamin C occurred during the first 55 days of storage. Similar results were found by [Giannakourou and Taoukis \(2003\)](#) for frozen green beans, spinach, green peas and okra, and by [Sahari et al. \(2004\)](#) for strawberries. [Gębczyński and Lisiewska \(2006\)](#) observed a significantly better vitamin C retention in broccoli stored at -30 °C.

An Arrhenius first order kinetic model [Eq. \(6\)](#) satisfactorily fitted the experimental data of vitamin C degradation under frozen storage (global fit of data obtained at all

temperatures in [Fig. 2a](#); kinetic parameters included in [Table 1](#); $R^2 = 0.96$).

The estimated activation energy for vitamin C degradation of frozen broccoli was higher than the ones obtained by [Martins and Silva \(2003\)](#) for green beans (42 kJ mol^{-1}) and by [Gonçalves et al. \(2009a\)](#) for watercress (25 kJ mol^{-1}). However, the values reported by [Giannakourou and Taoukis \(2003\)](#) for green peas, green beans, spinach and okra ranged from 98 to 112 kJ mol^{-1} .

Shelf-life of frozen broccoli was established taking into account the half-life of vitamin C (ascorbic acid) content ([Serpen et al., 2007](#)). Predictions based on [Eq. \(6\)](#) and parameters in [Table 1](#) allowed concluding that the shelf-life of pre-blanching frozen broccoli can achieve 125 days at -18 °C.

3.2.2. Non-isothermal storage conditions

[Fig. 2b](#) shows the changes in broccoli vitamin C (ascorbic acid) through the frozen storage at non-isothermal conditions. At the end of the storage period considered, broccoli losses c.a. 51% of its vitamin C content. Therefore, and using [Serpen et al. \(2007\)](#) criterion (reduction of 50% of vitamin C content), broccoli reached its shelf-life. Based on the same principle, [Giannakourou and Taoukis \(2003\)](#) reported shorten shelf-life for frozen green peas and spinach (25 and 5 days, respectively) exposed to changeable temperatures. Frozen raspberry stored for 24 days under temperature fluctuations decreased c.a. 20% of its initial vitamin C content ([Sousa et al., 2005](#)). However, [Cruz et al. \(2009\)](#) and [Gormley et al. \(2002\)](#) did not notice variation in vitamin C content in watercress and strawberries respectively, submitted to time-varying temperature conditions.

An Arrhenius first order kinetic model [Eq. \(8\)](#) was also satisfactorily fitted to experimental data for the degradation of vitamin C content (see [Fig. 2b](#)). The quality of the model fit was assessed by analyses to the residuals. The value of R^2 was 0.67. Estimated kinetic parameters are included in [Table 1](#). [Martins and Silva \(2004b\)](#) for green beans reported higher activation energy values ($41.97 \text{ kJ mol}^{-1}$).

3.3. Drip loss

During frozen storage, ice crystal recrystallization may cause loss of cell turgor following in a leakage of cell content and loss of cell water holding capacity, resulting in thawing drip loss. A consequence is a direct texture damaged and changes in the sensory crispness or juiciness of vegetable tissue and a discard in a significant amount of water-soluble nutrients ([Simandjuntak et al., 1996](#); [Van Buggenhout et al., 2006b](#)). Therefore, the amount of the thawing drip loss is an indicator of reversibility effect of the frozen process on the tissue, cells and fibres of vegetables ([Pukszta and Palich, 2007](#)).

3.3.1. Isothermal storage conditions

[Fig. 3a](#) shows the effect of the three different storage temperatures on frozen broccoli drip loss. As storage time and temperature increase, a significantly increase in drip loss was observed. During storage at -7 , -15 and -25 °C, drip loss increased approximately 88%, 43% and 12%, respectively, at the end of the storage period. It was the quality parameter mostly affected by the temperature of storage. The increase in drip loss is suggestive of a mechanical disruption of the cell

Fig. 2 – Broccoli vitamin C (ascorbic acid) content degradation during frozen storage: (a) under isothermal conditions (b) under non-isothermal conditions (dotted line indicates temperature history of regime II).

Fig. 3 – Broccoli thawing drip loss (%) during frozen storage: (a) under isothermal conditions; (b) under non-isothermal conditions (dotted line indicates temperature history of regime II).

wall promoted by changes in size, number and form of ice crystals (Hagiwara et al., 2002). Other authors have also reported the increase in drip loss with frozen storage time in different fruits and vegetables (Van Buggenhout et al., 2006a, O’Leary et al., 2000). However, for Redmond et al. (2004) frozen storage for up to 12 months had no statistically significant effect on the centrifugal drip loss from steamed freeze-chilling carrots and green beans. This quality parameter is widely used to evaluate different freezing methods on foods quality, or to assess the suitability of different vegetables cultivars to frozen storage (Fúster et al., 1994; Agnelli and Mascheroni, 2002; Lefever et al., 2004).

A zero order kinetic model with Arrhenius temperature dependence Eq. (5) fitted well the experimental data for drip loss (Fig. 3a). The activation energy and the reaction rate at a reference temperature of $-15\text{ }^{\circ}\text{C}$, and corresponding 95% confidence intervals, are reported in Table 1. The quality of the model fit was assessed by analysis to the residuals (i.e. normality and randomness were confirmed). The value of regression coefficient (R^2) was 0.94. The E_a estimated value

was lower than those obtained for the other quality parameters analysed (colour and vitamin C). No published data on drip loss kinetics have been found in the literature.

In addition, drip loss was negatively correlated with colour a^*/a_0^* parameter and vitamin C (ascorbic acid). The correlation coefficients of Pearson were 0.92 and 0.60, respectively.

3.3.2. Non-isothermal storage conditions

Under non-isothermal conditions, drip loss occurred at a significant faster rate when compared to isothermal regimes (Fig. 3b). At the end of storage (57 days), broccoli drip loss suffered an alteration of approximately 143.5%. Comparing this result with the worse scenario obtained under isothermal conditions (drip loss of 87% at the highest temperature of $-7\text{ }^{\circ}\text{C}$) it can be concluded that non-isothermal storage had a great impact. Puksza and Palich (2007) observed also a considerable increase in the amount of thawing drip loss (c.a 79%) for strawberries stored under time-varying temperature conditions.

The broccoli drip loss followed a first order reaction kinetics (8); experimental data and model fits in Fig. 3b; estimated parameters in Table 1), with a coefficient of determination of 0.75.

4. Conclusion

Frozen storage of broccoli under isothermal and non-isothermal conditions affected the colour, vitamin C (ascorbic acid) content and thawing drip loss. The accelerated life test method used in this work was a good tool for studying kinetics of quality changes.

Drip loss was the most sensitive quality factor to frozen storage under isothermal and non-isothermal condition. Applying a threshold of 50% vitamin C loss, as recommended, the shelf-life of frozen broccoli stored under a constant temperature of $-18\text{ }^{\circ}\text{C}$ was about 4 months.

To minimise oxidation processes in broccoli, alterations in colour and water migration phenomena, fluctuations of temperature throughout frozen storage and distribution should be controlled.

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