Kinetics of quality changes of pumpkin (*Curcubita maxima* L.) stored under isothermal and non-isothermal frozen conditions

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The effects of freezing process and frozen storage 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 on pumpkin quality were investigated. Storage temperature conditions were selected to embrace the limits practiced in the cold chain. Quality changes, such as texture, colour CIE *Lab* and vitamin C (ascorbic acid) content, were evaluated for both frozen storage regimes. The freezing process (that included a pre-blanching step) and subsequent frozen storage had significant impacts on all quality parameters analysed.

A fractional conversion kinetic model was adequate in colour, texture and vitamin C data fits. The storage temperature effect was successfully described by the Arrhenius law.

This study shows that non-isothermal frozen storage has a marked effect on pumpkin quality.

1. Introduction

Convenience and health issues are important factors for consumer's preferences when selecting frozen vegetables. Consumption of frozen products has increased over the past two decades, since the commodity of having available products out of season, conjoined with easy-to-cook forms, are appealing to consumers.

Freezing consists on lowering the temperature of the product till −18 °C (at the thermal centre), resulting in crystallization of most of the water and some solutes (Canet, 1989). It is desirable that frozen vegetables retain as much as possible fresh-like characteristics and, to attain this goal, process conditions must be conveniently designed.

A great number of factors may affect the final quality of frozen vegetables: characteristics of the raw material (Genin and Rene, 1996), pre-treatments applied before freezing (such as blanching; Song et al., 2003) and the freezing process itself (Sahari et al., 2004) are some examples.

Frozen storage is an integral and important part of frozen foods distribution chain. A significant number of recent studies reinforce the importance of post-processing temperature conditions and temperature fluctuations throughout storage and distribution, on rates of quality degradation and on shelf-life of frozen vegetables.

Generally, frozen foods are industrially stored at temperatures higher than −25 °C and are distributed near the limit of legally allowed ones (−18 °C, in most of the countries). At this temperature (−18 °C), water in vegetables tissues is not completely frozen. Only when the glass transition temperature is achieved, the vegetables are completely inert. This temperature depends on vegetables type, e.g. it is around −26 and −20 °C for young and old peas, respectively (Lim et al., 2006), and −32 °C for pumpkin (Gonçalves et al., 2007a). The glass transition temperature is a time dependent change on the physical state. This transition greatly influences food stability, as the water in the concentrated phase becomes kinetically immobilized and therefore does not support or participate in reactions (Rahman, 2006).

Since glass transition temperatures are generally lower than distribution temperatures, losses of quality during frozen storage may occur by chemical and/or physical means. The extent of these alterations depends on storage time–temperature conditions and product type. The major physical change that occurs during frozen storage is recrystallisation. Recrystallisation comprises changes in the number, size, shape, and orientation of the crystals, which are formed after initial solidification of the freezing phase, and it is the result of the successive melting on the surface of small ice crystals. This phenomenon has been verified in a wide range of foods, including vegetable tissues, and can be effectively controlled by storing products at a constant low temperature for a short time period.

The type of chemical changes that occur during frozen storage includes protein insolubilization, lipid hydrolysis and oxidation,

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natural pigment degradation, vitamin deterioration and brown pigment formation. Particularly in vegetables, textural changes throughout frozen storage can be attributed to structural alterations of protein membranes and to disruption of cellulosic cell walls by ice crystal growth (Powrie, 1984).

Temperature is therefore the main important factor for quality retention of frozen products throughout storage. However, and particularly during transportation and distribution of foods (Canet, 1989), deviations from ideal frozen storage temperatures occur quite often, as well as undesirable time-varying temperature conditions.

Comprehensive studies on the influence of frozen storage conditions on vegetables quality have been carried out using measurements of particular compounds/characteristics, such as ascorbic acid degradation, transformation of pigments, and alterations in sensory characteristics, like colour and texture. Studies on green beans, tomato, potato, strawberries and broccoli are some examples (Aparicio-Cuesta et al., 1989; Lisiewska and Kimiecik, 2000; Gormley et al., 2002).

Modelling the degradation kinetics of food components and nutrient losses during isothermal frozen storage has also received attention (Martins and Silva, 2002, 2003; Giannakourou and Taoukis, 2003; Gonçalves et al., 2009). These studies are decisively important, since the knowledge of kinetic parameters will allow products' final quality prediction and, consequently, improvements may be attained. Published quality changes kinetics of vegetables stored under frozen conditions are included in Table 1. However, some drawbacks may appear when experiments are designed for kinetic parameters estimation under static frozen conditions. The number of temperatures required (i.e. at least three for including temperature effects on kinetic parameters), and consequently the number of frozen chambers required for storage (one at each temperature), as well as the extensive storage times for quality changes assessment, are examples of experimental limitations. Alternatively, the use of accelerated life testing (ALT) overcomes these difficulties (Martins and Silva, 2004; Martins et al., 2005). The ALT on a product or material is used to get fast information on its life distribution. Experiments are performed at higher than usual stress conditions in order to induce rapid alterations. A model relating life length to stress is fitted to the accelerated failure times, and then is extrapolated to estimate the failure time distribution under usual conditions. The stress loading in an ALT can be applied in various forms. These include constant stress, step stress and random stress. Nelson (1990) and Martins and Silva (2004) discussed advantages and disadvantages of each application.

The objectives of this work were to investigate the freezing process (with a pre-blanching step) and frozen storage effects on some quality factors of pumpkin (*Curcubita maxima* L.). Kinetic parameters of quality changes were estimated isothermally (at -7 , -15 and -25 °C during 140 days of storage) and using accelerated life testing methodology (i.e. step-stress within the temperature range from -30 to -5 °C, throughout 57 days of storage). The results will allow assessment of both methodologies in estimation of frozen food quality losses. Relevant information about the adequacy of pumpkin vegetables to the frozen process will also be attained.

2. Materials and methods

2.1. Raw material and sample preparation

Pumpkins (*C. maxima* L.), in a fully ripe stage, were obtained in a local market in Lisbon, Portugal, 1 day after harvesting. Pumpkin fruits were immediately peeled and cut in cylinders of 50 mm diameter and 15 mm height, using a cork borer. Physical and biochemical raw pumpkin characteristics are numbered in Table 2.

2.2. Pre-blanching and freezing process

Samples (400 g of pumpkin cylinders each) were immersed in a thermostatic water bath (± 1 °C) with 50 L capacity, at optimised blanched condition of 95 °C during 3.9 min (conditions reported by Gonçalves et al., 2007b). After blanching, the samples were cooled in an iced water bath for 2 min. Excess of moisture was removed before any further analysis and/or process.

Random blanched samples were frozen in a vertical forced air freezer (Refriger, Electo-Refrigeração, Portugal) at -40 °C (average value), until the temperature on the centre of cylindrical 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 samples (~ 500 g each) were immediately packed into polyethylene bags (22×35 cm) and sealed. Representatives of blanched and frozen (prior to frozen storage) samples were analysed (see Table 2).

2.3. Frozen storage regimes

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

Table 1
Published quality changes kinetics of vegetables stored under isothermal and non-isothermal frozen conditions.

Frozen conditions	Product	Quality factor	Kinetic model	Kinetic parameters (Arrhenius equation)		References
				$k_{ref(T_{ref})} \times 10^3$ (days $^{-1}$)	E_a (kJ mol $^{-1}$)	
Isothermal	Watercress	Ascorbic acid	First order	$4.32_{[-15 \text{ } ^\circ\text{C}]}$	24.72	Gonçalves et al. (2009)
		Colour h^*	Zero order	$2.87_{[-15 \text{ } ^\circ\text{C}]}$	174.71	
	Green beans	Colour a	First order	$6.6_{[-15 \text{ } ^\circ\text{C}]}$	103.05	Martins et al. (2005)
		Colour b	First order	$22.2_{[-15 \text{ } ^\circ\text{C}]}$	55.33	
		Colour TCD	Fractional conversion	$6.0_{[-15 \text{ } ^\circ\text{C}]}$	106.27	
		Texture	Fractional conversion	$38_{[-15 \text{ } ^\circ\text{C}]}$	7.11	Martins et al. (2004)
		Ascorbic acid	First order	$32.3_{[-15 \text{ } ^\circ\text{C}]}$	42.01	
	Green beans	Ascorbic acid	First order	$2.23_{[-20 \text{ } ^\circ\text{C}]}$	101.5	Giannakourou and Taoukis (2003)
	Green peas		First order	$2.13_{[-20 \text{ } ^\circ\text{C}]}$	97.9	
	Spinach		First order	$4.54_{[-20 \text{ } ^\circ\text{C}]}$	112	
	Okra		First order	$1.05_{[-20 \text{ } ^\circ\text{C}]}$	105.9	
Non-isothermal	Green beans	Ascorbic acid	First order	$32.1_{[-15 \text{ } ^\circ\text{C}]}$	41.97	Martins and Silva (2004)
		Colour a	First order	$1.8_{[-15 \text{ } ^\circ\text{C}]}$	111.97	Martins et al. (2005)
		Colour b	First order	$12.1_{[-15 \text{ } ^\circ\text{C}]}$	75.29	
		Colour TCD	Fractional conversion	$136_{[-15 \text{ } ^\circ\text{C}]}$	140.34	

Table 2

Physical and biochemical characterisation of raw, blanched and frozen (prior to frozen storage) pumpkin.

Quality factor	Raw	Blanched	Frozen
<i>Texture</i>			
Firmness (N)	65.15 ± 7.82 ^a	52.55 ± 3.5 ^b	14.56 ± 1.71 ^c
Energy × 10 ³ (J)	74.78 ± 4.49 ^a	41.86 ± 2.51 ^b	25.69 ± 1.30 ^c
<i>Colour</i>			
<i>L</i> [*]	61.48 ± 1.39 ^a	51.27 ± 1.48 ^b	45.87 ± 1.21 ^c
<i>a</i> [*]	24.18 ± 2.10 ^a	15.30 ± 1.06 ^b	13.92 ± 0.91 ^b
<i>b</i> [*]	53.72 ± 1.74 ^a	39.14 ± 1.84 ^b	36.51 ± 2.28 ^b
Vitamin C (ascorbic acid) (mg/100 g)	15.46 ± 1.03 ^a	13.14 ± 0.95 ^b	10.99 ± 0.76 ^c
Peroxidase activity (Abs min ⁻¹ g ⁻¹)	2.19 ± 0.43 ^a	0.205 ± 0.03 ^b	0.019 ± 0.001 ^b

^{a,b,c} Values (in the same line) with different characters differ significantly ($P \leq 0.05$).

2.3.1. Regime I

Corresponds to isothermal frozen storage conditions at -7 , -15 and -25 °C (± 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 140 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 (geometric centre) 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.

2.4. Quality analysis

2.4.1. Microstructure

Preparation and fixation of samples for microstructure assessment were performed as described by Pinto et al. (2002). Tissue samples were transversally cut into pieces with a surgery thin blade. Samples were fixed with 2.0/100 mL glutaraldehyde in 0.04 mol/L of PIPES buffer pH 7.6 (Duchefa, Haarlem, The Netherlands) at 4 °C for 16 h. Dehydration was achieved by successive immersions in aqueous ethanol solutions (30–100/100 mL), acetone solutions (30–100/100 mL) and in a critical point device (Baltec CPD 030, Canonsburg, PA, USA) using CO₂ as transition agent. Samples were fixed on steel supports and coated with gold using a JEOL metalizer (FFC-1100, Tokyo, Japan) at 1100–1200 V and 5 mA for 10 min. Samples were observed in a scanning electron microscope (Hitachi, S4100, Tokyo, Japan) at 20 kV.

Raw, blanched and frozen (prior to the frozen storage) samples were analysed in triplicate.

2.4.2. Texture

Texture measurements were performed in a TA.HDI Texture Analyser (Stable Micro-System Ltd., Godalming, UK), using a 500 N load cell equipped with a 5 mm diameter probe. A single puncture measurement was made on each sample (10 mm depth of penetration, velocity of 1.0 mm s^{-1}). Force–distance curves were recorded and firmness (maximum peak force, N) and energy (area under force–distance curve, J) were used as indicator of textural parameters.

At least 12 measurements were done for each tested condition.

2.4.3. Peroxidase activity

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. (2007b).

Raw, blanched and frozen (prior to the frozen storage and at the last day of frozen storage) samples were analysed in triplicate.

2.4.4. Colour

Colour was assessed using a handheld tristimulus colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan) and a CIE standard illuminant C to determine CIE colour space co-ordinates, $L^*a^*b^*$ values. The parameter L^*a^*/b^* was calculated from the cartesian coordinates to describe frozen pumpkin alterations in colour. The total colour difference (TCD*) was the parameter considered for the overall colour difference evaluation:

$$\text{TCD}^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

where L^* , a^* and b^* are coordinates measured at time t , and L_0^* , a_0^* and b_0^* are the values obtained for the frozen samples, after defrost and before frozen storage.

The colorimeter was calibrated against a standard white reference tile. Samples were placed in a clear glass Petri dish (10 replicates), and colour measurements were done in triplicate.

2.4.5. Vitamin C

A modified titrimetric AOAC (1984) method was used for vitamin C (ascorbic acid) determination. Samples (200 g each) were blended with an equal weight of metaphosphoric acid (6%) using a Waring commercial blender (Dynamics Corporation, CT, USA). A 20 g of the macerate was added to 100 mL of metaphosphoric acid (3%) and filtered (Whatman no. 42 filter paper). The filtered sample (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 of ascorbic acid per 100 g of pumpkin sample.

2.5. Modelling procedures

2.5.1. The models

Under isothermal frozen conditions, kinetics of quality changes may be described by a fractional conversion model (Martins and Silva, 2003; Martins et al., 2005):

$$C = C_{eq} + (C_0 - C_{eq}) \exp(-kt) \quad (2)$$

where C is the measured quality factor (the indexes 0 and eq correspond to initial and residual values, respectively), t is the storage time and k the reaction rate.

The dependence of the reaction rate on temperature is commonly described by an Arrhenius equation (Giannakourou and Taoukis, 2003):

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

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 (3), thus giving:

$$C = C_{\text{eq}} + (C_0 - C_{\text{eq}}) \exp \left(-k_{\text{ref}} \exp \left(-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \right) t \right) \quad (4)$$

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

$$C = C_{\text{eq}} + (C_0 - C_{\text{eq}}) \exp \left[-k_{\text{ref}} \int_0^t \exp \left[-\frac{E_a}{R} \left(\frac{1}{T(t)} - \frac{1}{T_{\text{ref}}} \right) \right] dt \right] \quad (5)$$

The temperatures used for modelling procedures were the ones observed in the frozen environment, which did not significantly differ from the ones monitored at the geometric centre of the product.

2.5.2. Data analysis

The model parameters were estimated by non-linear regression analysis.

For isothermal conditions, Eq. (4) was fitted to experimental data using Statistica© 6.0 (StatSoft, 1999), 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. (5) was 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 assumed reference temperature was -15°C in all cases (i.e. the average value of the experimental range considered), aiming at improving parameters 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 (Hill and Grieger-Block, 1980), thus testing model adequacy.

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

3. Results and discussion

3.1. Pre-blanching and freezing process effects

Physical and biochemical characterisation of raw, blanched and frozen (prior to frozen storage) pumpkin samples is included in Table 2. Texture was significantly affected by blanching and freezing operations. When compared to raw samples, firmness of blanched pumpkin suffered a decrease of 19%. The freezing operation caused the most significant softening action on pumpkin tissues (i.e. firmness decreased 72% when samples are frozen after blanching).

Colour of pumpkin was also significantly affected ($P < 0.05$) by blanching pre-treatments. The samples became darker and lost redness and yellowness. However, the freezing process did not significantly affect a^* and b^* colour parameters (when compared to blanched samples).

Vitamin C (ascorbic acid) content was affected by blanching and freezing. A reduction of 29% was observed after these processes were sequentially applied. However, the magnitude of this degradation may be considered low, suggesting that processing

Fig. 1. Scanning electron micrographs of pumpkin cross section tissue at magnification 100× and observation at 15 kV: (a) raw sample; (b) blanched sample at 95 °C for 3.9 min; and (c) frozen sample.

conditions were adequate in minimising the freezing process impact on nutritional pumpkin quality. Bahçeci et al. (2005) reported decreases around 40% of ascorbic acid after green beans blanching, and Jaworska et al. (2008) reported decreases around 43% of vitamin C after blanching and freezing of agaricus bisporus.

Pre-blanching inactivated approximately 90% of initial POD activity. After freezing, POD presented only a residual activity. No regeneration of the enzyme activity was observed during frozen storage, for both temperature regimes. Similar results were also referred by Gonçalves et al. (2009) in watercress.

Microphotographs of raw pumpkin parenchyma (Fig. 1a) showed a well integrated tissue structure, composed by only one type of cells. In general, vegetables cells are round. However, and due to extrusion and collision, their shape usually became polyhedron after processing. These shapes can be observed in pumpkin cells where the bright regions in the micrograph are mainly cytoplasmic membrane and cell walls. The micrographs of blanched and frozen pumpkin are shown in Fig. 1b and c, respectively. For blanched samples, the cells appeared torn and irregular in shape and some loss of amorphous material and tissue distortion were observed. The tissue appearance of the frozen product indicates that freezing caused tissue shrinkage and cell collapse, supporting the lowest firmness value obtained for these samples (see Table 2).

3.2. Texture changes

3.2.1. Isothermal conditions

Both textural attributes analysed (firmness and energy) changed significantly ($P < 0.05$) with time at all conditions of both frozen regimes tested, and the higher temperature (-7°C) caused the faster rate of change. Fig. 2a shows the variation of energy textural attribute of pumpkin with frozen storage time, as well as the temperature impact. The variation of firmness was identical (data not shown). It can be observed that at -7°C and till the 80th day of storage, the energy decreased approximately 70% (in relation to the value observed prior to frozen storage) tending to an equilibrium value (around $5 \times 10^{-3}\text{J}$), which could be considered the pumpkin “frozen storage texture”. This designation was proposed by Alvarez and Canet (2000) and is indicative of the texture that resists to degradation caused by storage under frozen conditions.

A fractional conversion model (Eq. (4)) was adequate in describing the isothermal texture kinetics, both for firmness and energy (the model fit for energy is included in Fig. 2a). The adequacy was proven by the coefficient of determination ($R^2 = 0.92$ and 0.90 , for energy and firmness, respectively), and randomness and normality of the residuals. Parameters estimates and related precision, assessed by confidence intervals at 95%, are in Table 3.

Martins et al. (2004), besides reporting similar kinetics for texture degradation of frozen green beans, found considerable lower E_a values (see Table 1). The texture degradation rate, at the reference temperature, was lower for pumpkin than for green beans, which is indicative that texture of pumpkin is better retained.

3.2.2. Non-isothermal conditions

A rapid loss of texture was observed until the 20th day of frozen storage (curiously, this corresponds to the end of the first temperature step tested, i.e. -30°C), tending afterwards to an equilibrium value of approximately $16 \times 10^{-3}\text{J}$ (Fig. 2b). At the end of non-isothermal conditions storage (57 days), pumpkin texture suffered a decrease of 39% (in relation to the energy observed prior to frozen storage, i.e. $25.69 \times 10^{-3} \pm 1.30 \times 10^{-3}\text{J}$). Comparing this decrease to the one that occurred under isothermal storage for the same period of time, it can be concluded that it was higher than degradation observed at -25°C (26%), similar to the one obtained at -15°C (41%) and lower than the one occurring at -7°C (63%). Gormley et al. (2002), when studying texture changes of strawber-

Fig. 2. Pumpkin energy textural changes during frozen storage at: (a) isothermal conditions; and (b) non-isothermal conditions (dotted line indicates temperature history of regime II).

ries, concluded as well that when fluctuating temperature frozen regimes occurred, texture was better retained than when constant temperature frozen storage is applied.

Under non-isothermal conditions, the fractional conversion model (Eq. (5)) was also adequate in describing texture changes throughout frozen storage (the model fit for energy is included in Fig. 2b). The coefficient of determination was 0.76 , and randomness and normality of the residuals were verified. Parameters estimates are in Table 4. In this situation the confidence intervals were undetermined, due to the singularity of the hessian matrix (Bates and Watts, 1988).

The kinetics of pumpkin texture degradation under non-isothermal conditions presented considerably lower activation energy values, when compared to isothermal conditions. However, under non-isothermal conditions texture is better retained in long term storage (assessed by the equilibrium values estimated).

No reported texture kinetic data related to frozen vegetables at non-isothermal conditions was found in the literature.

3.3. Colour losses

3.3.1. Isothermal conditions

Colour CIE L^*a/b^* parameter changes occurred at significant faster rates, particularly at the highest storage temperature tested

Table 3

Kinetic parameters, and corresponding confidence intervals at 95%, of pumpkin texture, colour and vitamin C degradation, due to isothermal frozen storage.

Quality factor		Kinetic parameter				
		C_0	C_{eq}	$k_{-15\text{ }^{\circ}\text{C}} \times 10^3 \text{ (days}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	R^2
Texture	Firmness (N)	11.68 ± 0.19	5.05 ± 0.36	9.04 ± 1.35	90.61 ± 10.83	0.90
	Energy $\times 10^3$ (J)	22.59 ± 0.48	4.77 ± 1.10	11.91 ± 1.80	43.02 ± 3.75	0.92
Colour	L^*a^*/b^*	15.72 ± 0.46	3.56 ± 0.51	21.70 ± 3.0	35.66 ± 3.58	0.93
	TCD*	0	17.55 ± 1.31	19.0 ± 4.00	24.86 ± 4.97	0.85
Vitamin C (ascorbic acid)	mg/100 g	9.74 ± 0.52	2.89 ± 0.47	25.50 ± 6.40	41.39 ± 7.22	0.93

Table 4

Kinetic parameters of pumpkin texture, colour and vitamin C degradation, due to non-isothermal frozen storage.

Quality factor		Kinetic parameter				
		C_0	C_{eq}	$k_{-15\text{ }^{\circ}\text{C}} \text{ (days}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	R^2
Texture	Energy $\times 10^3$ (J)	22.78	15.76	8.84×10^{-2}	1.93	0.76
Colour	TCD*	0	24.16	7.85×10^{-2}	2.01×10^{-6}	0.60
Vitamin C (ascorbic acid)	mg/100 g	10.64	1.18×10^{-6}	1.47×10^{-2}	3.18	0.85

($-7\text{ }^{\circ}\text{C}$) (data not shown). Frozen storage promotes degradation on carotenoids compounds due to isomerization or oxidation changes, leading to loss of pigments that cause fading of pumpkin orange colour characteristic and loss of its nutritive value (Dutta et al., 2005).

Fig. 3a presents the pumpkin TCD* changes as affected by storage at different temperatures, during 140 days. Significant alterations were observed at all temperatures. At the end of frozen storage TCD* was 19.6, 14.5 and 9.8 (average of experimental observations) at -7 , -15 and $-25\text{ }^{\circ}\text{C}$, respectively.

An Arrhenius fraction conversion kinetics model (Eq. (4)) was satisfactorily fitted to experimental data for both L^*a^*/b^* and TCD* (see Fig. 3a for TCD* behaviour and estimated parameters and regression analyses results in Table 3). For both colour parameters, normality and randomness of residuals were verified, and the coefficients of determination were satisfactorily high (0.93 and 0.85 for L^*a^*/b^* and TCD*, respectively).

The E_a estimated value for TCD* is lower than the one reported by Martins et al. (2005) for frozen green beans (Table 1). At the reference temperature, colour of frozen pumpkin degrades in a faster rate than green beans.

3.3.2. Non-isothermal conditions

Despite a great variability of characteristics occur in biological tissues, which is the case of carotenoids content associated to pumpkin colour, it is possible to say that under non-isothermal conditions colour alterations occurred at significant faster rates when compared to isothermal regimes within the same temperature range (Fig. 3b). The estimated equilibrium value was 24.16, corresponding to very great differences (according to the classification scale of Drlange (1994)). Under isothermal frozen storage, the equilibrium was significantly lower, revealing that higher colour retention was attained.

The adequacy of the fractional conversion model was verified (i.e. residuals randomness and normality), being R^2 equal to 0.60. Estimated model parameters are included in Table 4. The activation energy for this process is considerably low ($2.01 \times 10^{-6} \text{ kJ mol}^{-1}$).

3.4. Vitamin C degradation

3.4.1. Isothermal conditions

Fig. 4a shows the effect of temperature on vitamin C (ascorbic acid) content of pumpkin, during frozen storage under isothermal conditions. It can be observed that vitamin C was more stable at

lower temperatures, as expected. More than half of the pumpkin vitamin C content was rapidly deteriorated during the first 20th days of storage at $-7\text{ }^{\circ}\text{C}$; for $-25\text{ }^{\circ}\text{C}$, 120 days were necessary to

Fig. 3. Pumpkin colour degradation during frozen storage: (a) TCD* at isothermal conditions; and (b) TCD* at non-isothermal conditions (dotted line indicates temperature history of regime II).

degrade the same amount. If these results are compared with those published by Giannakourou and Taoukis (2003) for green beans, spinach, green peas and okra, pumpkin seems to be a very sensitive product to frozen storage.

During isothermal frozen storage, vitamin C loss followed a fractional conversion Arrhenius temperature dependent kinetics (Eq. (4); kinetic parameters and confidence intervals included in Table 3; $R^2 = 0.93$). Martins and Silva (2004) reported similar values related to vitamin C degradation of frozen green beans. However, for pumpkin a residual value of vitamin C (2.89 ± 0.47) is observed. Giannakourou and Taoukis (2003) found higher E_a values for green peas, green beans and spinach, and Gonçalves et al. (2009) reported lower values for watercress (Table 1). These differences may be due to the composition and structure of food systems. Subtle differences in water activity, pH, metal ions, sugars content and state of bioactive constituents may have significant influence on rate and/or mechanism of ascorbic acid degradation and therefore in the activation energy involved.

3.4.2. Non-isothermal conditions

Fig. 4b shows the changes of pumpkin vitamin C content throughout the tested regime of frozen storage under non-isothermal conditions. Based on nutritional criteria (50% of vitamin C degradation), the shelf-life of pumpkin was 50 days. At the end of the

storage (57 days), pumpkin degraded approximately 73% of its initial vitamin C content. In the last 7 days at -5°C , pumpkin loses $2.42\text{ mg}/100\text{ g}$ (average value). Sousa et al. (2005) reported also that fluctuations in temperature influence ascorbic acid content of raspberry fruits.

Vitamin C (ascorbic acid) degradation due to time-varying temperature conditions was well described by the fractional conversion kinetic model (Eq. (5); kinetic parameters included in Table 4; $R^2 = 0.85$). The estimated activation energy was 3.18 kJ mol^{-1} . For green beans, Martins and Silva (2004) reported higher values.

4. Conclusions

The freezing process and frozen storage at isothermal and non-isothermal conditions generated quality losses of pumpkin, reflected in texture, colour and vitamin C degradation.

Under non-isothermal frozen storage, lower E_a values were obtained for quality factors degradation, when compared to the isothermal situation within the same temperature limits. The ALT method was a good tool for obtaining foods quality loss kinetics, while reducing experimental storage times in shelf life studies, once the degradation mechanisms are well known. The model obtained in this study can be valuable in predicting time of degradation of a quality factor using temperatures within the studied range.

Shelf-life threshold of frozen pumpkin can be established using vitamin C content as a standard indicator of quality stability of frozen vegetable. If the isothermal kinetic model is used, a threshold of 50% of vitamin C content is estimated if pumpkin is stored at -18°C for 61 days; if the non-isothermal model is used, the shelf-life prediction is 58 days.

Overall it can be concluded that frozen pumpkin should be stored at constant and low temperatures.

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Fig. 4. Pumpkin vitamin C (ascorbic acid) degradation during frozen storage at: (a) isothermal conditions; and (b) non-isothermal conditions (dotted line indicates temperature history of regime II).

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