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Kinetic modelling of vitamin C loss in frozen green vegetables under variable storage conditions

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Abstract

A systematic kinetic study of L-ascorbic acid loss of four green vegetables was conducted in the temperature range of freezing storage. The temperature-dependence of vitamin C loss in the −3 to −20 °C range was adequately modelled by the Arrhenius equation and activation energy ranged from 98 to 112 kJ/mol for the four frozen green vegetables. The developed models were validated in fluctuating time–temperature conditions, in order to establish their applicability in the real marketing path of the commercial products. Based on the models, the nutritional level can be estimated, at any point of the freezing chain, when the full time–temperature history is available. Comparison among different green vegetables showed that the type of plant tissue significantly affects the rate of vitamin C loss. Frozen spinach was found to be the most susceptible to vitamin C degradation, peas and green beans demonstrated a moderate retention, whereas okra exhibited a substantially lower loss rate.

Keywords: Frozen vegetables; Shelf life; Arrhenius; Distribution

1. Introduction

Vegetables, an important component of a balanced human diet, are low in fat, low in energy with high carbohydrate and fibre contents, providing significant levels of some micronutrients. Fresh vegetables have a short durability, and are exposed to conditions that destroy their superior quality in a short period of time, before cooking and consumption. Seasonality and perishability of vegetables explain the necessity of applying preservation technologies, such as freezing. The aim is to combine shelf life extension with maintenance of sensory and nutrient characteristics. The main factors affecting the final quality of frozen vegetables are: raw material, processing, including blanching treatment and method of freezing, and post-processing distribution, storage and home-handling (Labuza, 1982). Post-processing temperature conditions and temperature fluctuations determine the rate of quality degradation and the shelf life of frozen vegetables. Improper frozen storage causes evident changes in sensory characteristics that can influence consumer acceptability, but also leads to products of reduced nutritive value, mainly in vitamin C.

A considerable body of work on the different modes of quality degradation of different frozen vegetables has been published and reviewed in the earlier and recent literature (Hung & Thomson, 1989; Jul, 1984; Kramer, 1974; Labuza, 1982; Martens, 1986; Martins & Silva, 1998; Pilar Cano, 1999). The current effort is to develop and apply a systematic kinetic and modelling approach to the main quality indices of each product. By establishing the appropriate quality function that describes the time-temperature dependence of the selected mode of degradation, a quantitative tool for shelf life estimation and management is obtained (Giannakourou, Skiadopoulos, Polydera, & Taoukis, 2001).

Vegetables are a major source of ascorbic acid, a nutrient that besides its vitamin action is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported, such as inhibition of formation of cancer-causing N-nitroso compounds in the stomach (Hussein et al., 2000; Sanchez-Mata, Camara-Hurtado,

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Diez-Marques, & Torija-Isasa, 2000). During processing, distribution, and storage of frozen vegetables, ascorbic acid oxidizes to dehydroascorbic acid, DHAA, which is irreversibly hydrolysed to 2,3 diketogulonic acid, which possesses no vitamin C activity. This oxidation is enhanced by temperature abuses during frozen storage. The retention of ascorbic acid in frozen products is thus strongly dependent on their temperature history (Favell, 1998; Makhlouf, Zee, Tremblay, Belanger, Michaud, & Gosselin, 1995). The level of vitamin C, besides being an indicator of nutrient value, can be used, in the case of frozen vegetables, as a reliable and representative index for estimating the quality deterioration at any point of the marketing route of a product to its final destination, the consumer. Recent studies report vitamin C contents of several frozen green vegetables (Favell, 1998; Haag, Ylikoski, & Kampulainen, 1995; Howard, Wong, Perr, & Klein, 1999; Kmiecik & Lisiewska, 1999; Lisiewska & Kmiecik, 1996, 1997; Oruña-Concha, Gonzalez-Castro, Lopez-Hernandez, & Simal-Lozano, 1998), and the effect of pretreatments and storage temperatures on the preservation of ascorbic acid. They include the nutritional content at steady, low temperature, indicative of frozen practice (e.g at −20 and −30 °C), at the beginning, in the middle and at the end of their commercial shelf-life, without assuming a full, systematic kinetic approach. The temperature range in most studies does not cover the −3 to −10 °C range which is very detrimental and does frequently occur in the real frozen chain (e.g. European Union survey EE1080/94/00069, 1995; Giannakourou & Taoukis, in press). Additionally, the applicability of shelf-life models, under possible temperature fluctuations, has not been fully addressed. In order to be able to predict, in a reliable way, at any point of its life cycle, the nutritional level of a product, based on its temperature history, it is important that the established kinetic equations cover the whole relevant range of temperatures and are validated in dynamic, non-isothermal conditions. Another issue to be considered is that reactions in frozen foods, mainly due to the freeze-concentration effect in the immediate subfreezing temperature and the glass to rubber transition that occurs at T_g (Goff, 1997; Kerr, Lim, Reid, & Chen, 1993; Roos, 2001; Taoukis, Labuza, & Saguy, 1997) may show a temperature dependence that deviates from the Arrhenius law. The effect of glass transition (T_g) on the quality and the shelf life of frozen products has recently received considerable attention (Andersen & Skibsted, 1998; Roos, 1995; Roos, Karel, & Kokini, 1996). It has been proposed that the quality kinetics near the T_g could be alternatively described by the Williams–Landel–Ferry (WLF) model (Maltini & Anese, 1995; Nelson & Labuza, 1994; Roos & Karel, 1991), correlating food stability directly with the temperature difference between storage temperature and T_g. However, its applicability, as an alternative to the Arrhenius approach, is debatable and has to be further examined with appropriate kinetic data (Manzocco, Nicoli, Anese, Pitotti, & Maltini, 1999; Roos & Himberg 1994).

The objective of this work is the development of kinetic models of the nutritional quality (vitamin C content) of four widely consumed frozen green vegetables over the entire temperature range of practical interest. These kinetic equations are validated in variable conditions, in order to be used for the reliable prediction of the remaining shelf life of the product at any point of their route to the consumer, based on nutritional criteria and the full temperature history of the product. A comparative assessment of vitamin C loss rates in the different vegetable matrices tested and of the application of the established kinetic equations is conducted.

2. Materials and methods

2.1. Frozen vegetable samples and experimental design

The studied frozen vegetables were green peas (*Pisum sativum,* variety Karina and Geneva), spinach (*Spinacia oleracea*), green beans (*Phaseolus vulgaris, L)* and okra (*Abelmoschus esculentus)*. Industrial processing includes prefreezing treatments, such as rinsing and blanching at 90 °C for 2 min, and cooling, followed by the freezing process in a freezing tunnel (IQF freezing equipment, FloFreeze MA-Model, Frigoscandia, Helsingborg, Sweden) at −22 °C for 2 min, packing and storage in the factory warehouses at −18 °C. The final products were transported to the laboratory under carefully monitored conditions, ensuring minimal quality loss. Samples of 100 g of green vegetable were packed in the same laminate film (20 μm BOPP–48 μm PE) used for the commercial products and were stored in controlled temperature cabinets (Sanyo MIR 153 and 253, Sanyo Electric Co, Ora-Gun, Gunma, Japan) at constant temperatures (from −1 to −20 °C) or programmed variable temperature profiles, constantly monitored by type T thermocouples and a multichanel datalogger (CR10X, Campbell Scientific, Leicestershire, UK). Samples were obtained at appropriate time intervals for each storage temperature based on ASLT (Accelerated Shelf Life Testing) methodology (detailed by Taoukis et al., 1997), and a rough estimate of expected temperature-dependence of vitamin C loss rate from previously published data.

2.2. Vitamin C determination

Vitamin C was determined by a high performance liquid chromatography method (HPLC), which was compared and standardized with the 2, 6 dichloroindophenol titrimetric method (AOAC, 1984, 43.064). All analyses were carried out in duplicate on vegetable tissue, homogenized, using a pestle and mortar (Oruña-Concha et al., 1998). Five grammes of homogenate were mechanically stirred in 15 ml of a 4.5%(w/v) solution of metaphosphoric acid for 15 min. The mixture was vacuum-filtered and diluted with HPLC grade water; the total final volume was measured and an aliquot was filtered through a 0.45-μm Millipore filter prior to injection into the chromatographic column. The instrumentation details were: HP Series 1100 (quaternary pump, vacuum degasser, a Rheodyne 20-μl injection loop and a Diode-Array Detector, controlled by HPChemStation software); Hypersil ODS column (250×4.6 mm) of particle size 5 μm; mobile phase: HPLC grade water with metaphosphoric acid to pH 2.2; detection at 245 nm; calibrated by external standard method.

2.3. Data analysis

The results of vitamin C content, obtained for all vegetables in question, were plotted vs time for all temperatures studied, and the apparent order of L-ascorbic acid oxidation was determined. The temperature-dependence of the deterioration rate, k, was then modelled by the Arrhenius equation [Eq. (1)]:

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

where k_{ref} is the reaction rate of the vitamin C oxidation at a reference temperature T_{ref}, E_{A} is the activation energy of the chemical reaction and R is the universal gas constant. By linearly correlating lnk vs $(1/T_{\mathrm{ref}} - 1/T)$ (Arrhenius plot), the E_{A} of L-ascorbic oxidation was estimated from the slope of the fitted line.

An alternative way to describe the temperature-dependence of vitamin C loss in frozen vegetable matrices, as mentioned earlier, is the application of Williams–Landel–Ferry (WLF) kinetics, due to possible deviations from the Arrhenius law [Eq. (2)]:

$$\log\frac{k_{\mathrm{ref}}}{k} = \frac{-C_1(T - T_{\mathrm{ref}})}{C_2 + (T - T_{\mathrm{ref}})} \quad (2)$$

where C_1 and C_2 are system-dependent coefficients.

3. Results

3.1. Initial vitamin C variation

The content of vitamin C in the frozen green vegetables studied, just after freezing and prior to freezing storage, is listed in Table 1. The non-homogeneous nature of plant materials is reflected on the significant variability of the initial nutritional value, and no means of correlation with controlling factors, such as the cultivar, environmental parameters, or the maturity at the harvesting period is possible, due to no requirements for full traceability of the product. The lack of homogeneity within a batch (Crispin & Varey, 2002) is highlighted in Fig. 1, where the wide range of initial vitamin C content for frozen green peas of the same batch is plotted as a distribution curve. This demonstrates the need for caution in assuming a single fixed value for the initial vitamin C content. Nevertheless, mean averages show that the nutritional value does not differ much for the different species of the green vegetables in question. These results are in agreement with previous reports, as far as the relative order of nutritional content is concerned (Albrecht, Schafer, & Zottola, 1990, 1991; Favell, 1998), with spinach being slightly superior and green beans having the least initial nutritional value, after freezing. Albrecht et al. (1990) attributed the high initial vitamin C content of spinach to a direct correlation with the sulfur content, proposing an enzyme-catalyzed reaction in which reduced glutathione (a sulfur-containing tripeptide) reduces dehydroascorbic acid to ascorbic acid in spinach chloroplasts.

3.2. Kinetic study of vitamin C loss

The average retention of ascorbic acid is expressed relatively to an initial, average value of day 0 of the experiment (Figs. 2a, 3a, 5a and 5b), where C represents the concentration of ascorbic acid in 100 g of raw material. In all cases, vitamin C loss was found to be adequately described by an apparent first order reaction [Eq. (3)]:

$$C = C_0 e^{-kt} \quad \text{or} \quad \ln\frac{C}{C_0} = -kt \quad (3)$$

where C and C_0 are the concentrations of L-ascorbic acid at time t and zero, respectively, and k is the apparent reaction rate of vitamin C loss, estimated by the slope of the linearized plot of $\ln(C/C_0)$ vs t. Specifically, for each vegetable, the observations and results are described below.

After the freezing/blanching process, during the subsequent isothermal frozen storage, green peas and leafy spinach exhibited a first order loss of vitamin C at all temperatures studied (Figs. 2a and 3a). Temperature-dependence of vitamin C deterioration was expressed with the Arrhenius equation (Fig. 2b for the case of green peas), and the estimated activation energies, E_{A}, the 95% confidence range as well as the goodness of fit (R^2) and the estimated Q_{10} values for the range −15 to −5 °C, are shown in Table 2. The kinetic results for green peas (E_{A} = 97.9±9.6 kJ/mol) were different from the corresponding values estimated by Giannakourou and Taoukis (2002), where peas of variety Pudget were studied, demonstrating the possible effect of cultivar on

Table 1
Initial L-ascorbic acid for frozen spinach, green peas, green beans and okra

	Initial content of L-ascorbic acid (mg/100 g of frozen vegetable)			
	Green peas	Spinach	Green beans	Okra
Measured				
Mean value ± standard deviation	28.5±4.9	31.1±4.8	25.3±9.9	28.0±7.8
Number of samples	32	18	15	15
Range	(17–41)	(25–34)	(16–39)	(13–34)

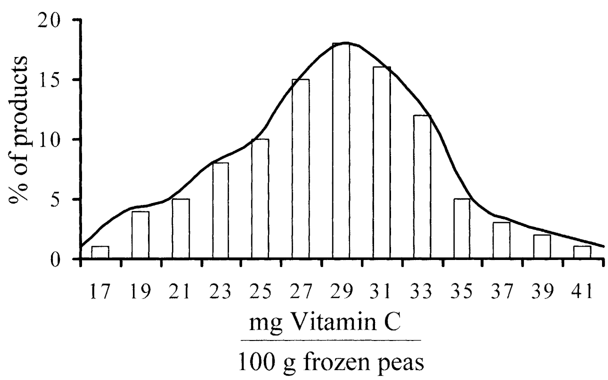

Fig. 1. Distribution of the initial content of L-ascorbic acid in frozen green peas.

the retention of vitamin C during frozen storage. In Fig. 3(b), the temperature effect is depicted by a shelf life plot for the case of frozen spinach. Shelf life values from earlier studies on the shelf life of frozen spinach (Kramer, 1974) could lead to different estimations of E_A, which may be attributed to different cultivars, that could affect the mode of deterioration, due to different enzyme concentration, water activity and other factors.

Apart from the previous analysis, an alternative approach for modelling the temperature dependence of the deterioration reaction was also applied, namely the WLF equation [Eq. (2)]. WLF model ($T_g' \cong -25$ °C and $T_{ref} = -20$ °C), shown in Fig. 4 for green peas, gives an adequate fit ($R^2 = 0.957$) but the estimated coefficient values ($C_1 = -3.5$ and $C_2 = 35.4$) were out of the range reported in the literature as WLF values are in the rubbery state. In the case of spinach, application of WLF (with $T_{ref} = -20$ °C) in the rubbery range resulted in the calculation of the coefficients $C_1 = -12.8$ and $C_2 = -147$ ($R^2 = 0.976$), outside the expected range reported in the literature (Nelson & Labuza, 1994). These results do not support any preference for the WLF model over the Arrhenius one in the temperature range tested for either of the two tissues studied. The Arrhenius approach was deemed adequate to represent temperature-dependence of ascorbic acid degradation within the rubbery state of frozen vegetable matrices.

Following the same methodology, a thorough kinetic study was also conducted for the other two commercial frozen green vegetables, green beans and okra. vitamin C content of green bean (initial value: 25 mg/100 g) exhibited a substantial first order reduction even when stored at low freezing temperatures (Fig. 5a). Okra, on the other hand, showed a comparatively high stability of vitamin C with significantly lower first order loss rates at all storage temperatures (Fig. 5b). For both products, the Arrhenius equation adequately expressed the tem-

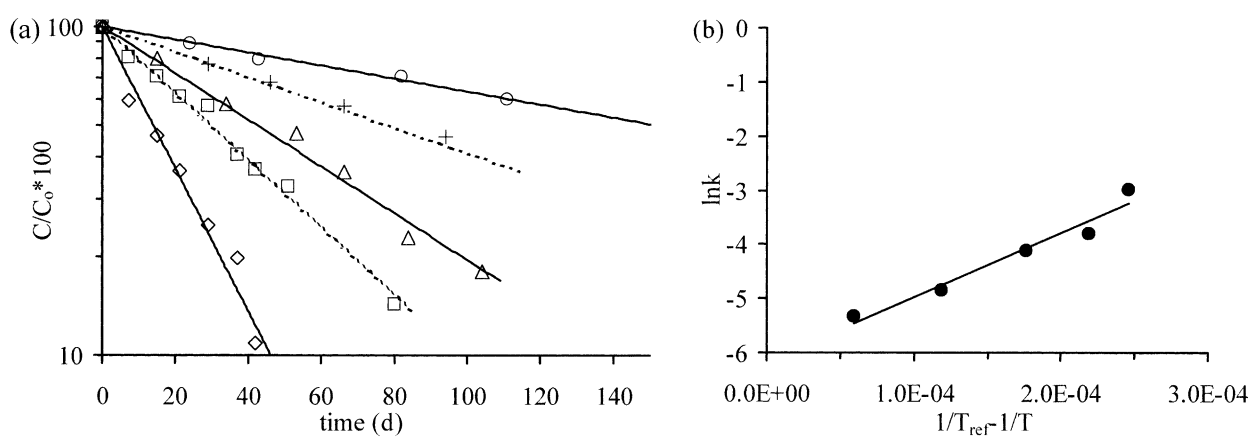

Fig. 2. (a) Results for vitamin C loss vs time at five storage temperatures, on a semilogarithmic scale. Experimental points correspond to: ◇ at −1 °C, □ at −3 °C, Δ at −8 °C, + at −12 °C and ○ at −16 °C and lines represent the first order fit (R^2 >0.980 at all temperatures). (b) Arrhenius plot of the vitamin C loss rate for frozen green peas (with $T_{ref} = -20$ °C).

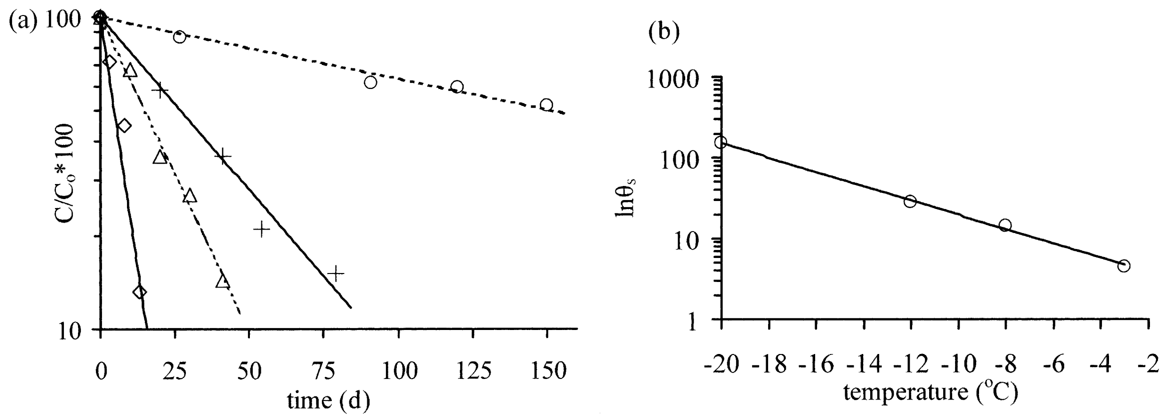

Fig. 3. (a) Results for vitamin C loss vs time at four storage temperatures on a semilogarithmic scale. Experimental points correspond to: ◇ at −3 °C, Δ at −8 °C, + at −12 °C and ○ at −20 °C and lines represent the first order fit (R^2 >0.972 at all temperatures). (b) Shelf life plot of the 50% vitamin C loss for frozen spinach on a semilogarithmic scale.

Table 2
Arrhenius parameters and statistics, Q_{10} values and shelf life, at four temperatures in the frozen storage range for frozen green vegetables

	Kinetic parameters			
	Green peas	Spinach	Green beans	Okra
E_A (kJ/mol)	97.9±9.6[a]	112±23.2	101.5	105.9
k_{ref} (1/d)	0.00213	0.00454	0.00223	0.00105
R^2	0.958	0.992	0.967	0.868
Q_{10} (in the range −15 to −5 °C)	5.5	7.0	5.8	6.3
Temperature	Shelf life (days)[b]			
−5 °C	24	8	21	40
−10 °C	56	20	50	98
−15 °C	132	55	122	249
−20 °C	325	153	311	660

[a] 95% Confidence intervals based on the statistical variation of the kinetic parameters of the Arrhenius model (regression analysis).
[b] Shelf life is based on 50% vitamin C loss.

perature-dependence of vitamin C loss, and the estimated activation energies E_A are shown in Table 2. In Fig. 6, the shelf life plots for both tissues illustrate the superior nutritional stability and the extended shelf life of okra. The application of WLF (with $T_{ref}=-20$ °C)

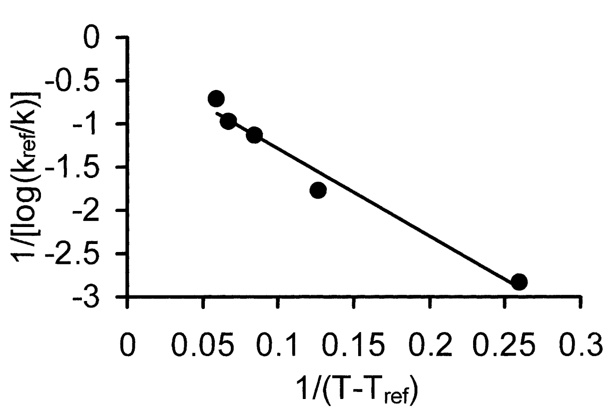

Fig. 4. WLF plot for the rate constant of the vitamin C loss in frozen green peas, with $T_{ref}=-20$ °C.

resulted in the calculation of the coefficients $C_1=4.56$ and $C_2=41.1$ for green beans ($R^2=0.942$) and $C_1=-7.12$ and $C_2=-89.3$ for okra ($R^2=0.986$). Although the fit was better than for the Arrhenius model in the case of okra, the estimated values for WLF constants were not in the expected range.

The established kinetic models were validated under dynamic storage conditions in programmable freezer-incubators. To demonstrate the integrated effect of the temperature variability on product quality, the term of the effective temperature T_{eff} is introduced. T_{eff}, which is defined as the constant temperature that results in the same quality value as the variable temperature distribution over the same time period, is based on the Arrhenius model and integrates, in a single value, the effect of the variable temperature profile. The nutritional change under variable temperature conditions $T(t)$ for time t_{tot} can be calculated by Eq. (4).

$$\ln\left(\frac{C_{t_{tot}}}{C_o}\right) = \int_0^{t_{tot}} k(T(t))\mathrm{d}t = k_{eff}t_{tot} \quad (4)$$

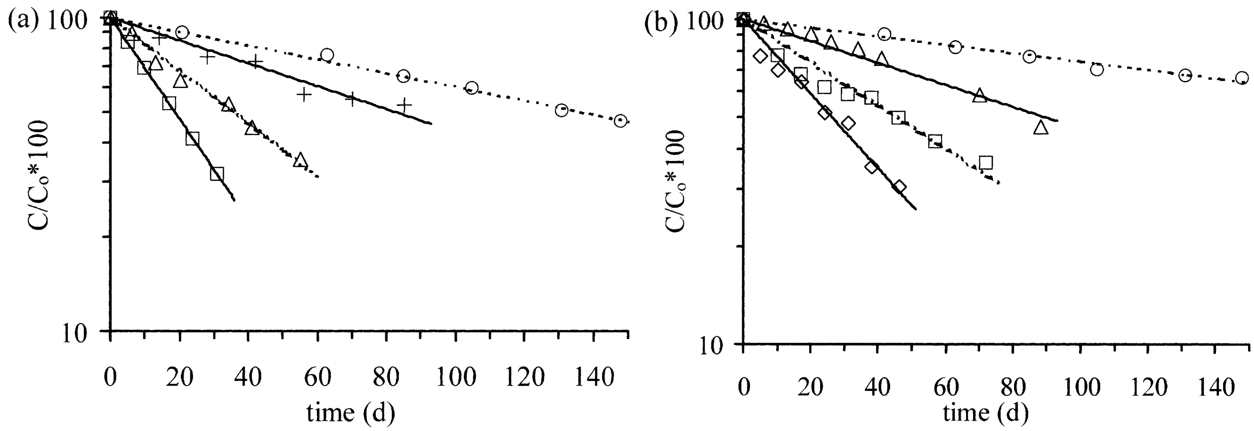

Fig. 5. Results for vitamin C loss vs time at four storage temperatures on a semilogarithmic scale. Experimental points correspond to: (a) □ at 5 °C, Δ at −8 °C, + at −12 °C and ○ at −16 °C, for frozen green beans and (b) ◇ at 3 °C, □ at −5 °C, Δ at −8 °C and ○ at −16 °C for frozen okra. Lines represent the first order fit ($R^2 > 0.930$ at all temperatures).

where k_{eff} is the value of the rate of vitamin C loss at the effective temperature. If the temperature profile is a step sequence, as in our experiments, or is separable into small time increments t_i of constant temperature T_i, where $\Sigma t_i = t_{\mathrm{tot}}$, then Eq. (4) can equivalently be expressed as:

$$k_{\mathrm{ref}} \sum_i \left(\exp\left[-\frac{E_A}{R}\left(\frac{1}{T_i} - \frac{1}{T_{\mathrm{ref}}}\right)\right] t_i \right) = k_{\mathrm{eff}} t_{\mathrm{tot}} \tag{5}$$

from which k_{eff} can be estimated. For $k = k_{\mathrm{eff}}$, the value of the effective temperature T_{eff} can be calculated from the Arrhenius equation [Eq. (1)].

In Figs. 7 and 8, measurements of vitamin loss and the corresponding exponential fit are shown and compared to predictions at the corresponding T_{eff}, with the dotted lines representing the limits of 95% confidence range of the quality prediction for green peas and spinach, respectively. Repeated temperature cycles inclu-

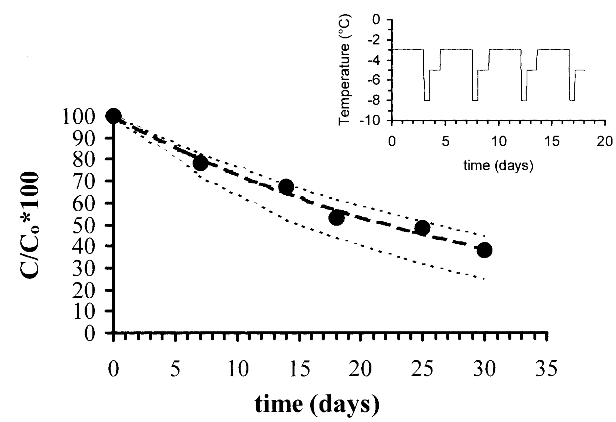

Fig. 7. Comparison of experimental (closed circles) and predicted results of vitamin C loss of frozen green peas for exposure at the shown variable temperature profile. The solid line represents the exponential fit of the quality measurements and dotted lines depict the upper and lower 95% confidence range of quality predicted for T_{eff}.

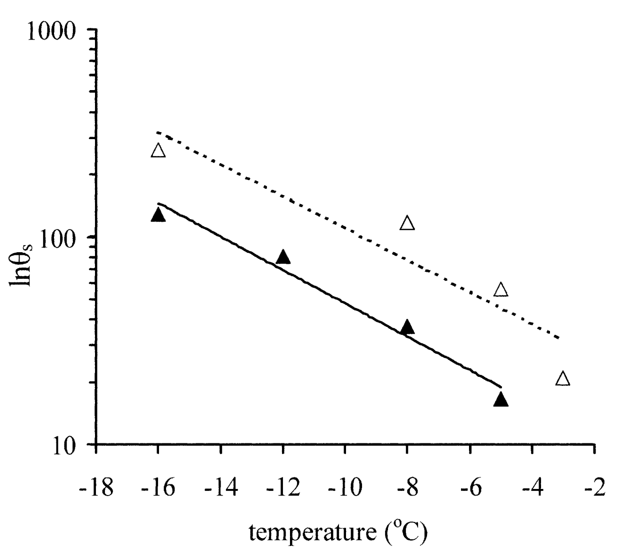

Fig. 6. Shelf-life plot of 50% vitamin C loss for frozen green beans (black triangles) and okra (open triangles).

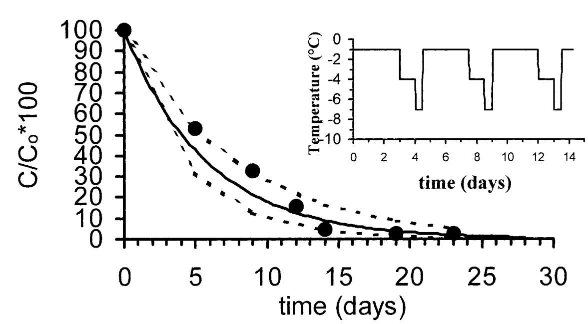

Fig. 8. Comparison of experimental (closed circles) and predicted results of vitamin C loss of spinach for exposure at the shown variable temperature profile. The solid line represents the exponential fit of the quality measurements and dotted lines depict the upper and lower 95% confidence range of quality predicted for T_{eff}.

Table 3
Time–temperature conditions of the repeating cycles and comparison of experimental (k_{exp}) with predicted (k_{eff}) rate of vitamin C loss for the non-isothermal experiments conducted for frozen green peas and spinach

	Green peas	Spinach
Time–temperature conditions of repeating cycles	1st stage: 72 h at −3 °C 2nd stage: 24 h at −5 °C 3rd stage: 12 h at −8 °C	1st stage: 72 h at −1 °C 2nd stage: 24 h at −4 °C 3rd stage: 12 h at −7 °C
T_{eff} (°C)	−3.8 °C	−1.7 °C
k_{eff} (1/d)	0.0349±0.0083[a]	0.1726±0.0427[a]
k_{exp} (1/d)	0.0315±0.0059[b]	0.1640±0.0545[b]
R^2 for k_{exp}	0.981	0.917

[a] 95% Confidence intervals based on the statistical variation of the kinetic parameters of the Arrhenius model (regression analysis).
[b] ±95% Confidence limits of experimental data (regression analysis).

ded three step changes, as shown in Figs. 7 and 8. The exact time–temperature sequences used are listed in Table 3. Predicted rates of loss, k_{eff}, are in good agreement with the experimentally estimated ones, k_{exp}, as is demonstrated in Table 3, where the 95% confidence intervals of k_{eff}, the goodness of fit and the ±95% confidence range of k_{exp} (t-test) are also calculated. Since the estimated values of k_{exp} fall within the 95% confidence intervals of k_{eff}, the two rates are considered statistically equivalent in both non-isothermal experiments conducted for the two different green vegetables.

Based on the validated kinetic models, the shelf life of green peas at different temperatures in the frozen range, based on nutritional criteria, is shown in Table 2. It is noteworthy that, in the case of spinach, the short shelf life at all temperatures, clearly demonstrates the sensitivity of this vegetable when compared to the other studied products.

4. Discussion

The main aspects of this study were the establishment of reliable kinetic models of vitamin C loss for green vegetables during frozen storage and the comparative estimation of their shelf life under dynamic temperature conditions, in the range of interest (−3 to −20 °C).

Green vegetables, after harvest, preparation, blanching and industrial quick freezing, are not immediately available (Favell, 1998) to the consumer. Often, several months may elapse before purchase, and a subsequent time period in the domestic freezer before final consumption, with fluctuating temperature conditions, often deviating from the ideal ones. Therefore, the design of this work sought to develop and validate kinetic models that would allow estimation of the rate of vitamin C loss during non-isothermal handling of products, mimicking the real distribution path of frozen foods. With the validated kinetic equations, the remaining shelf life of the vegetables in question could be predicted at any point of their distribution, from manufacture to consumption. For this purpose, a realistic distribution scenario (Fig. 9) in the current chill chain is assumed, that includes an initial stage of 10 days storage in the factory warehouses, intermediate transport, followed by 10 days' stocking at the wholesale stage (or alternatively in a distribution centre). Subsequently, vegetables are transported and exposed at retail freezers (either closed vertical or open horizontal freezers) for 20 days, before being purchased by the final consumers, that keep them stored for 20 days before final cooking and consumption. Temperature data for the initial stages are obtained from Jul (1984) and from the European survey EE/1080/94/00069 (1985), whereas temperatures in domestic freezers were recorded during a survey realized by our laboratory in 100 random home freezers (Giannakourou & Taoukis, 2003). When the time-temperature handling of products is constantly monitored, it is possible to estimate the extent of nutritional deterioration and the fraction of shelf life consumed, f_{con}, at the end of each distribution phase (Table 4). To calculate vitamin C loss after each distribution phase, the value of the corresponding k_{eff} and T_{eff} are estimated for the particular temperature profile from Eqs. (5) and (1). To calculate f_{con}, the fraction of shelf life consumed at each stage, the time/temperature/tolerance (TTT) approach (Labuza & Fu, 1997; Van Arsdel, Coply, & Olson, 1969) can equivalently be used.

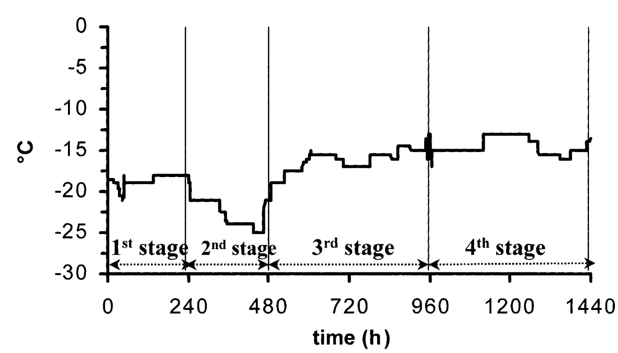

Fig. 9. Indicative temperature profile of distribution of frozen vegetables in the real chill chain (total distribution time 60 days).

Table 4
Vitamin C loss (%) and fraction of shelf life consumed, f_{con}, of frozen green vegetables at the end of each stage of the distribution cycle, illustrated in Fig. 9

	1st stage duration: 10 days $T_{eff} \cong -18.5$ °C		2nd stage duration: 10 days $T_{eff} \cong -22.3$ °C		3rd stage duration: 20 days $T_{eff} \cong -16.1$ °C		4th stage duration: 20 days $T_{eff} \cong -14.4$ °C	
	% Vit. C loss	f_{con}	% Vit. C loss	f_{con}	% Vit. C loss	f_{con}	% Vit. C loss	f_{con}
Okra	1.4	0.020	2.7	0.040	10.9	0.166	25.6	0.426
Peas	2.7	0.040	5.4	0.080	20.4	0.329	43.9	0.837
Beans	2.9	0.042	5.6	0.084	22.2	0.361	47.8	0.938
Spinach	6.0	0.092	11.1	0.170	41.3	0.767	75.8	1.039

The f_{con} is the sum of the times at each constant temperature segment, t_i, divided by the shelf life at that temperature, θ_i:

$$f_{con} = \sum \frac{t_i}{\theta_i} \tag{6}$$

where index i represents the different time–temperature steps within the particular stage. The remaining shelf life of these vegetables, at a reference temperature of e.g. -20 °C, can be calculated after each stage as $(1-\Sigma f_{con})*\theta$, where θ is the shelf life at -20 °C. At the end of the retail storage and exposure, the remaining shelf lives for spinach, green beans, green peas and okra are 36, 198, 218 and 549 days, respectively. At the end of their entire marketing route (60 days after production), at the time of consumption, spinach lost more than 50% vitamin C, under the specific temperature conditions, whereas green beans, peas and okra retained an acceptable nutritional quality (19, 53 and 378 days of remaining shelf life, respectively).

Comparing the relative retentions of vitamin C, the data showed a significant deviation in the deterioration rates of L-ascorbic acid for the different vegetables studied, with spinach having the most prominent sensitivity, followed by green peas and beans (Table 2). These observations are in line with previous reports (Albrecht et al., 1990; Favell, 1998), showing poor retention for spinach and moderate stability for green beans and green peas. This differing behaviour regarding vitamin C oxidation can be attributed to the different tissue structure, mechanical damage during harvesting, intrinsic enzyme (ascorbate oxidase) and sulphydryl group content, and the presence of metal ions, such as Fe^{3+} and Cu^{2+}, that act as catalysts. The loss of ascorbic acid is probably enhanced by the activity of ascorbate oxidase, which is strongly dependent on the pH of the vegetable. The latter explains the vulnerability of green beans that, although not suffering from mechanical damage during harvest, show subsequently significant nutritional loss, due to the high enzyme activity, and possibly low sulphydryl content. Peas, protected in their pods, and okra, having a tight structure, more adequately retain vitamin C. In contrast, spinach leaf is found to be very prone to rapidly lose its high initial vitamin C, due to its comparatively high surface area and to an elevated amount of iron, that is known to play a decisive role in the oxidative degradation of L-ascorbic acid to L-dehydroascorbic acid.

In this study, shelf life calculation, refers merely to nutritional (vitamin C) degradation. This is an important quality index, but the fact that commercial and consumer acceptability are mainly based on sensory quality criteria, such as colour, flavour, texture and juiceness, should not be overlooked. In the case of nutritional labelling, however, shelf life dating is "legally" limited by nutrient declaration and thus, can become the shelf-life determining criterion. In all cases, vitamin content can be used as a reliable index that reflects the whole temperature history of the product. Validated kinetic models of vitamin C and other quantifiable important quality loss indices for frozen vegetables, can be used for evaluation, control and proper management of the frozen chain, with the application of suitable time–temperature indicators (Giannakourou & Taoukis, 2002), and an appropriate correlation scheme.

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