

Effect of cold chain temperature abuses on the quality of frozen watercress (*Nasturtium officinale* R. Br.)

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A B S T R A C T

The objective of this work was to study the effect of temperature abuses on the colour and vitamin C content of a new frozen vegetable, watercress (*Nasturtium officinale* R. Br.).

The vitamin C content, expressed as ascorbic (AA) and dehydroascorbic (DHAA) acids, and colour, expressed in the Hunter *Lab* parameters, were evaluated along a plan of temperature abuses, based on a real situation for a four month frozen storage period. A comparison between the hue angle and AA experimental data and prediction models, using kinetic parameters determined under isothermal conditions, was also run.

The colour showed some variation, namely on the parameters *L*, *a*, TCD (Total Colour Difference) and hue angle. Although, during the temperature abuses, some fluctuation was observed, no vitamin C degradation or major colour changes occurred. A zero-order and a first-order prediction models fitted well the experimental data for the hue angle and AA behaviour with temperature abuses, respectively. In general, the frozen watercress colour and vitamin C content were not impaired by the imposed temperature abuses. This work will help to predict and understand the sensory and nutritional quality changes of frozen watercress that might occur during frozen storage and distribution in the cold chain under the used conditions.

Introduction

Watercress (*Nasturtium officinale* R. Br.) is a leafy vegetable of the family Cruciferae that grows in and around water. It is a good source of essential vitamins and minerals and beneficial phytochemicals, such as lutein and zeaxanthin (U.S. Department of Agriculture, 2003). Normally consumed fresh, it has a short shelf life (approximately seven days) that can be extended throughout freezing, allowing a longer period for distribution and storage.

Blanching is one of the pre-treatments used before freezing, to inactivate the enzymes and prevent biochemical reactions in the frozen product that might contribute for the development of off-flavours and discoloration (Mountney and Gould, 1988). However, blanching causes undesirable changes in the food properties that may result in loss of colour, flavour, texture and nutrients (Pala, 1983; Pizzocaro et al., 1995).

The freezing process is an excellent method to preserve taste, texture and nutritional value of foods, and better than most other preservation methods. Nevertheless, such qualities depend upon the careful choice of food materials, suitable pre-treatments,

choice of freezing process, and appropriate packaging and frozen storage options (Flair flow Europe, 2000; Blond and Le Meste, 2004).

Exposure to higher temperatures and/or fluctuations of storage temperature produces cumulative adverse effects on the quality of stored foods, which is the primary cause of damage to food marketed through retail channels (Blond and Le Meste, 2004).

The loss of quality is caused by physical and chemical changes taking place in the product. During the storage period, physical changes that affect frozen vegetables and fruits quality are a result of recrystallization and sublimation phenomena, related to the stability of the ice crystals inside and on the surface of the products. Recrystallization and surface drying are accelerated by temperature fluctuations during freezing, although the importance of these physical changes decreases at lower storage temperatures (Canet, 1989; Alvarez and Canet, 1998; Sousa et al., 2005).

New frozen product formulations and new freezing technologies benefits should be assessed taking into account the frequent temperature abuses occurring in commercial and domestic storage (Estrada-Flores, 2002).

In the distribution of frozen food, the cold chain starts at the raw material supplier and continues until the consumers' freezer, playing a very important role in the maintenance of food safety and quality. Therefore, in each major stage of the cold chain,

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Nomenclature

<i>a</i>	colour co-ordinate, represents red to green
<i>b</i>	colour co-ordinate, represents blue to yellow
<i>C</i>	parameter concentration or value
E_a	activation energy (kJ mol^{-1})
<i>k</i>	reaction rate (day^{-1})
<i>L</i>	colour co-ordinate, represents black to white
<i>R</i>	universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
<i>t</i>	time (days)

<i>T</i>	absolute temperature (K)
TCD	total colour difference parameter

Subscripts

<i>T</i>	relative to a given temperature
0	initial value at time equal to zero
ref	at the reference temperature

temperature requirements must be taken into account. Usually, the suggested storage temperature is below -18°C , in which the microbial growth is completely stopped, and both enzymatic and non enzymatic changes continue but at much slower rates during frozen food shelf life, although temperature of -15°C is permitted for short periods during transportation or local distribution. Moreover, food retail display cabinets should be at -18°C with good storage practice, but not warmer than -12°C . At the consumer's domestic freezers, storage temperature is also important, being the freezer 'star rating' related to its freezing capacity. A one-star freezer is capable of temperatures below -6°C , a two-star freezer of temperatures below -12°C , and a three-star freezer is capable of temperatures below -18°C (Flair flow Europe, 2000).

The objective of this work was to study the effect of temperature abuses on colour and vitamin C content of frozen watercress and with the findings help to understand the sensory and nutritional quality changes that might occur during frozen storage and distribution in the food chain.

Materials methods

Vegetable material

Raw watercress (*N. officinale* R. Br.) was kindly supplied by Vitacress, a company that grows watercress in Almancil, Algarve-Portugal. The leaves were directly harvested from the producer and, on the same day, processed (selected, washed, blanched and frozen).

Blanching and freezing processes

The leaves of watercress were blanched in a boiling pan (Armfield 45 L, Hampshire, England) in a proportion of 30 g per litre of water, set at 95°C during 20 s, and cooled in an iced water bath (Cruz et al., 2006). After the blanching process, the leaves (30 g) were pressed in a mould into a slab shape ($4.6 \times 3.3 \times 1.8 \text{ cm}$). The slabs were frozen in an air blast freezer (Armfield FT 36, Hampshire, England) at an average temperature of -25°C and an air velocity of 8 m s^{-1} , until the temperature of -18°C was reached at the centre of the sample. Samples triplicates for each step of analysis were packed in low density polyethylene bags and stored at -21°C (Haier HF-248, Germany). All temperatures were monitored with a digital thermometer (Ellab ctd 87, Roedovre, Denmark) and a thermocouple (1.2 mm needle dia; constantan-type T).

Temperature abuses plan

A plan of temperature abuses was established based on a real situation for a four month period (Oliveira et al., 2009) and applied to the frozen watercress packages (Fig. 1). The samples were stored in freezers (Haier HF-248, Germany) and analysed in each step of the plan (S1–S6 corresponds to production and distribution; S7–S20 corresponds to consumer product purchase and storage). The

watercress temperature was measured with a type T thermocouple placed in the slab thermal centre and recorded with a data acquisition system (Delta-T Devices DL2 e, Cambridge, England).

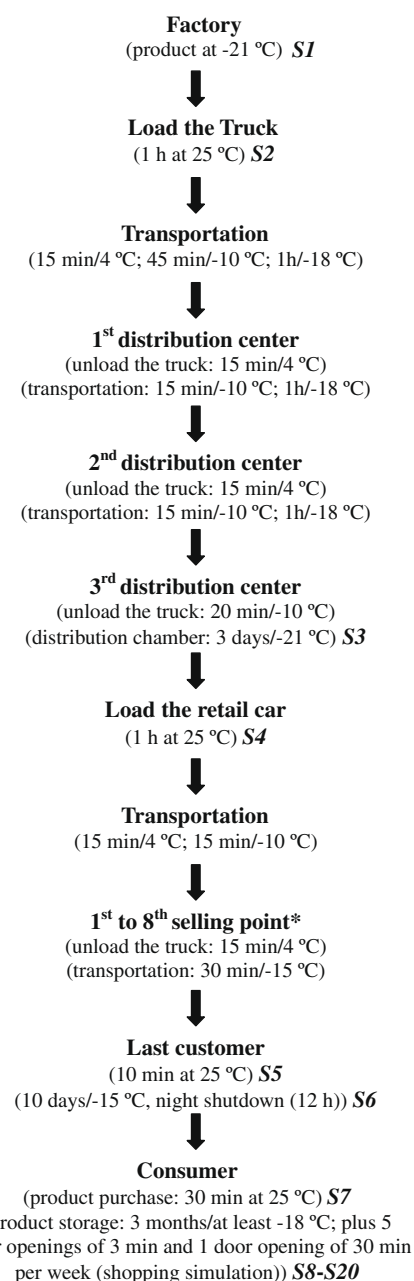


Fig. 1. Plan of temperature abuses. S1 to S20- steps of analysis; * These two steps were repeated eight times.

Colour was evaluated in terms of *Lab* values, with a tristimulus colorimeter (Dr Lange Spectro-colour, Berlin, Germany) in the Hunter system (HunterLab, 2000). The colorimeter (d/8° geometry, illuminant D65, 10° observer) was calibrated against a standard ceramic white tile ($X=84.60$, $Y=89.46$ and $Z=93.85$) and a standard ceramic black tile ($X=4.12$, $Y=4.38$ and $Z=4.71$). The colour changes were interpreted by calculating the TCD (Total Colour Differences) (Eq. (1)) where ΔL , Δa and Δb are determined by Eq. (2). The hue angle (tone or tint) was determined by Eq. (3) and chroma (saturation) by Eq. (4). Measurements were taken in triplicates.

$$\text{TCD} = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

$$\begin{aligned} \Delta L &= L - L_0 \\ \Delta a &= a - a_0 \\ \Delta b &= b - b_0 \end{aligned} \quad (2)$$

where L_0 , a_0 and b_0 are the readings of the frozen samples before temperature abuses, and L , a and b the individual readings at each step of the temperature abuses plan.

$$\text{Hue angle} = (\tan^{-1} b/a) \quad (3)$$

$$\text{Chroma} = (a^2 + b^2)^{1/2} \quad (4)$$

Ascorbic acid and dehydroascorbic acid analysis

Ascorbic acid (Riedel-de Haën) content was determined based on a method previously reported (Zapata and Dufour, 1992), by reverse phase ion interaction high performance liquid chromatography HPLC UV detection using isoascorbic acid (IAA) (Fluka) as internal standard. Dehydroascorbic acid (Sigma) was as well detected as fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one (DFQ), after pre-column derivatization with 1,2-phenylenediamine dihydrochloride (OPDA) (Sigma).

For the mobile phase, 13.61 g of potassium dihydrogen phosphate (Merk) and 3.64 g of cetrimide (Fluka) were added to 2 l of methanol-ultrapure water (5:95, v/v). The eluent was filtered in a 0.45 μm membrane (Macherey-Nagel, Porafil) and degassed in an ultrasonic bath for 15 min. The HPLC system consisted of a controller (LKB-2152, Bromma, Sweden), a solvent pump (LKB-2150, Bromma, Sweden), an injection valve with a 20 μl sample loop, a guard pre-column (Macherey-Nagel, Chromcart Nucleosil 100-10 C18) followed by a reversed phase column (Macherey-Nagel, Chromcart 100-10 Nucleosil, 250 \times 4.6 mm) and a UV detector (LKB-2153, Bromma, Sweden).

Each sample was homogenized with an Ultra-turrax (IKA T25 Janke & Kunkel, Staufen, Germany) in 20 ml of methanol-ultrapure water (5:95, v/v) for 5 min at 8000 rpm. Then, 5 ml were transferred to a 20 ml volumetric flask and 1 ml of IA standard solution (0.03 g/50 ml) was added. The pH was adjusted with HCl (Merk) to obtain final values between 2.20 and 2.45. The volume was completed to 20 ml with methanol-ultrapure water (5:95, v/v). The content was centrifuged (Sigma 3 K20, Osterode, Germany) for 5 min at 8.720 \times g and 4 °C. Afterwards, 3 ml were transferred to another tube with 1 ml of OPDA (Sigma) (0.03 g/50 ml) daily prepared and maintained in dark. The mixture was then vortexed and placed in the dark at room temperature for 40 min. Then the mixture was filtered in a 0.45 μm membrane (Millipore), the first millilitre was discarded and 20 μl were injected in the HPLC. The wavelength detector was set to 348 nm and after elution of DHAA, the wavelength was shifted to 262 nm for AA and IA detection. The experiments were run in triplicate.

Tissue microstructure was only examined to evaluate the effect of the blanching process. Thus, after blanching, the leaves were stored in a vertical freezer at -80 °C (Snijders Scientific, Tilburg, The Netherlands). Afterwards, based on the method reported by Fonseca et al. (2005), the samples were transversally cut with a surgery thin blade and observed in a scanning electron microscope (SEM) (JEOL JSM-5600 LV, Tokyo, Japan) at low vacuum with an acceleration voltage of 15 kV and a cryo-chamber set at -25 °C. The experiments were run with six replicates.

Prediction of ascorbic acid and hue value

The ascorbic acid and hue angle experimental data were compared with prediction models (using the kinetic parameters estimated under isothermal conditions (Table 2) (Gonçalves et al., 2009), in which the integration of the temperature variability with time was required.

The reaction rate k_T , for which the temperature dependence followed the Arrhenius behaviour, was calculated using Eq. (5):

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

Where k_{ref} is the reaction rate at the reference temperature, E_a , the activation energy (previously determined under isothermal conditions), R , the universal gas constant, T , the absolute temperature, and T_{ref} , the reference temperature (258.15 K).

In the isothermal study, a first-order reaction (Eq. (6)) fitted well the ascorbic acid experimental data. While in the colour evaluation, the parameters were estimated from a zero-order reaction (Eq. (7)) (Gonçalves et al., 2009).

$$\frac{C(t)}{C_0} = \exp(-k_T t) \quad (6)$$

$$C(t) = C_0 - k_T t \quad (7)$$

Under non-isothermal conditions, since $T=f(t)$, the ascorbic acid and hue angle values can be predicted, for a given time, by Eqs. (8) and (9), respectively.

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

$$C(t) = C_0 - k_{\text{ref}} \int_0^t \exp \left[-\frac{E_a}{R} \left(\frac{1}{T(t)} - \frac{1}{T_{\text{ref}}} \right) \right] dt \quad (9)$$

Statistical analysis

Calculations were performed using the statistical software SPSS 17.0. One-way analysis of variance (ANOVA) was carried out to determine if there were significant differences between each step of the plan of temperature abuses. The Least Significant Difference (LSD) test was run to determine the significant differences between

Table 1
Watercress colour and vitamin C content before the plan of temperature abuses.

	Fresh	Blanched	Frozen
<i>L</i>	35.36 \pm 0.37	31.02 \pm 0.91	29.05 \pm 2.53
<i>a</i>	-10.35 \pm 0.03	-10.47 \pm 0.17	-4.58 \pm 0.74
<i>b</i>	12.30 \pm 0.16	9.05 \pm 0.21	3.97 \pm 1.31
Chroma	16.08 \pm 0.14	13.84 \pm 0.25	6.08 \pm 1.39
Hue angle (°)	130.07 \pm 0.29	139.16 \pm 0.43	139.94 \pm 5.89
AA (mg 100 g fw ⁻¹)	35.66 \pm 1.42	4.51 \pm 0.48	4.63 \pm 0.77
DHAA (mg 100 g fw ⁻¹)	29.17 \pm 8.31	6.32 \pm 1.06	8.61 \pm 5.17
Total vitamin C (mg 100 g fw ⁻¹)	64.83 \pm 7.85	10.83 \pm 0.58	13.25 \pm 4.43

Table 2

Published watercress AA and hue value kinetic parameters under isothermal conditions (Gonçalves et al., 2009).

Kinetic parameters	AA	Hue angle (°)
E_a (kJ mol ⁻¹)	24.73	174.71
$k_{ref - 15\text{ °C}}$ (d ⁻¹)	0.00432	0.00287
C_0	32.62 mg 100 g ⁻¹	132.35
Kinetic model	First-order	Zero-order

the control (without abuses) and each treatment. Evaluations were based on a significance level of 5%.

Results and discussion

Quality parameters

The experimental values for watercress colour and vitamin C content before the plan of temperature abuses are shown in Table 1. The applied blanching process, aiming 90% reduction of the enzyme peroxidase, caused some colour changes and degradation on the vitamin C content. From Fig. 2, it can be observed that, after blanching, the *L* and *b* parameters decreased about 12% and 26%, respectively, showing that the samples became darker and less yellow. On the other hand, the *a* parameter did not change after the blanching treatment. Colour changes after blanching could be related with the replacement of the gases inside the intercellular spaces by the blanching medium, altering light refraction from the cell surface (Bowers, 1992).

In terms of microstructure quality, the fresh sample (Fig. 3a and a') showed the parenchyma cells and vascular tissue turgid and well defined. The blanched watercress microstructure (Fig. 3b and b') presented the parenchyma cells filled with spherical structures (probably chloroplasts released from inside the cells). The blanched samples also showed a more damaged microstructure, being the tissue firmness and cell wall strength rapidly lost due to the heat extent.

Under thermal blanching, the cell wall membrane may be damaged and allow water to enter the cell. Internal organelles may be distorted and begin to leak their contents. A reduction of cell turgidity is caused by the loss of cell membrane function. Moreover,

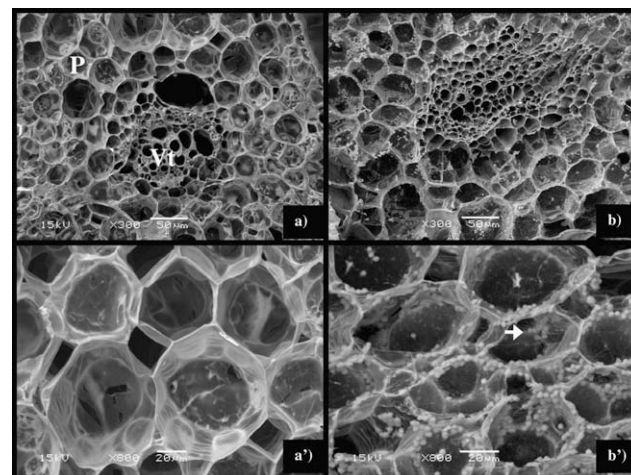


Fig. 3. SEM micrographs of watercress cross section tissue at different magnification levels (300× and 800×): (a and a') Fresh watercress; (b and b') Blanched watercress. P = parenchyma cells, Vt = vascular tissue, white arrow indicate the chloroplasts.

pectic substances are released from the cell wall. This causes less cohesion between adjacent cells and thus a breakdown of the structure (Kerr, 2004).

Throughout the plan of temperature abuses the results showed some variations ($P < 0.05$) in the *L* parameter between the samples with, and samples without, temperature abuses (Fig. 2). The samples with six, seven, twelve and thirteen weeks of storage, showed the highest differences comparing with the non abused samples. The lightness variations were probably due to the ice crystals formation or water loss along the temperature abuses.

The *b* parameter showed no significant differences ($P > 0.05$) between the control and all the steps until the end of the plan, meaning that the yellowness was not affected by the temperature fluctuations (Fig. 2).

Although in the *a* parameter, significant differences ($P < 0.05$) were detected between the control and the S2 step (1 h at 25 °C), the observed experimental data during the plan of temperature abuses did not reveal any trend, stabilising during the consumer product storage.

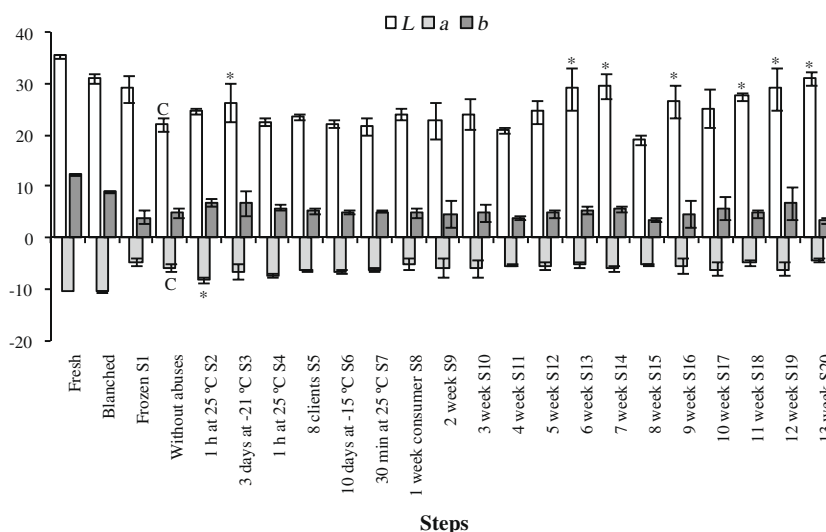


Fig. 2. Hunter Lab parameters for frozen watercress along the temperature abuses storage. Bars represent mean ± standard deviation from three replicates. C – control; * significant differences with control ($\alpha = 0.05$).

The TCD values showed some fluctuations along the plan with an average value of 4 (Fig. 4). These results are in agreement with those reported by Gormley et al. (2002) in which frozen strawberries submitted to temperature fluctuations showed values of TCD in the same range.

The chroma decreased after freezing about 50% (less saturated) and showed no significant differences ($P > 0.05$) between all the steps throughout the temperature plan (Fig. 4).

The hue angle (Fig. 5) increased from fresh to blanched watercress suggesting that the samples became greener after processing, corroborating the observed a parameter values. Along the temperature abuses, the samples showed values between 135–145° and significant differences ($P < 0.05$), only between the control and the S13 step (sixth week at the consumer).

Fig. 6 presents the ascorbic and dehydroascorbic acids and total vitamin C contents throughout the storage plan. The results showed some fluctuations ($P < 0.05$) in both active forms. In terms of dehydroascorbic acid, the product purchase step showed the

lowest value observed ($P < 0.05$). This result was probably due to the temperature increase and the consequently conversion of the dehydroascorbic acid to 2,3-diketogulonic acid.

The watercress total vitamin C also showed some fluctuation. The variation throughout the temperature abuses plan is mainly due to the temperature fluctuations. However, that variation can also be associated to the watercress vitamin C initial variability. Nevertheless, the vitamin C value from the beginning until the end of the plan was practically maintained.

In a work reported by Rácz and Szabolcs (1978), with spinach purée under the conditions of the distribution chain, the vitamin C content decreased with storage time and temperature. However, the watercress results in this work are in agreement with those reported by Gormley et al. (2002), in which the vitamin C of frozen strawberries, submitted to temperature fluctuations, was practically maintained when compared to the control.

Sousa et al. (2005) verified in a study with frozen raspberry that temperature is a much more important factor than storage time in

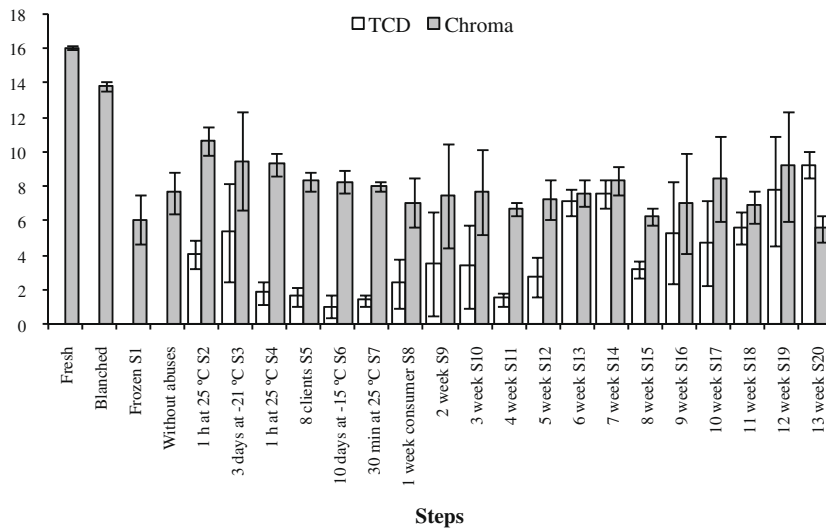


Fig. 4. TCD and chroma parameters for frozen watercress along storage with temperature abuses. Bars represent mean \pm standard deviation from three replicates.

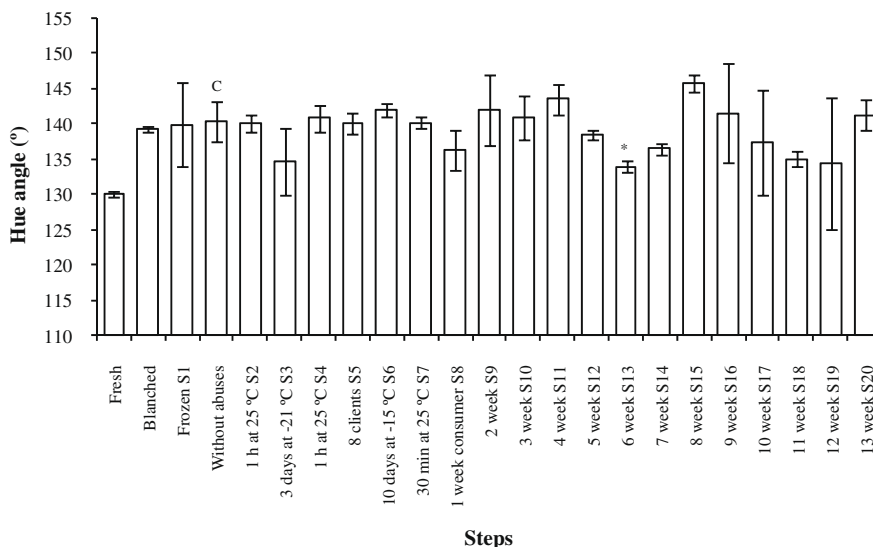


Fig. 5. Hue parameter for frozen watercress along storage with temperature abuses. Bars represent mean \pm standard deviation from three replicates. C – control; * significant differences with control ($\alpha = 0.05$).

determining quality loss. Short term frozen storage with fluctuations caused a decrease in the ascorbic acid content of the raspberry fruits.

In a study with frozen spinach at constant temperature, samples stored at -29°C during one year showed ascorbic acid losses of 10%, while at -12°C the initial content decreased 50–75% (Watt, 1977).

The retention of ascorbic acid in frozen products is thus strongly dependent on their temperature history. 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 (Gianakourou and Taoukis, 2003; Fennema, 1977).

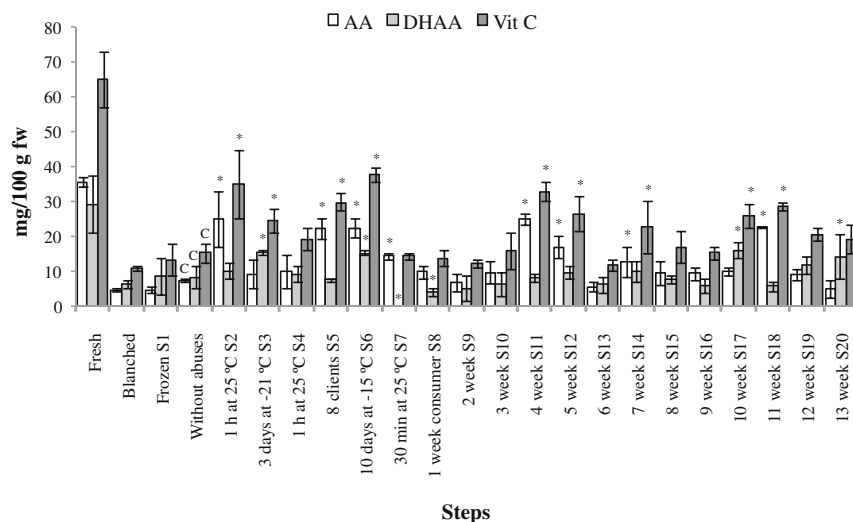


Fig. 6. Ascorbic acid, dehydroascorbic acid and total vitamin C evolution along the temperature abuses plan. Bars represent mean \pm standard deviation from three replicates. C-control; significant differences with control ($\alpha = 0.05$).

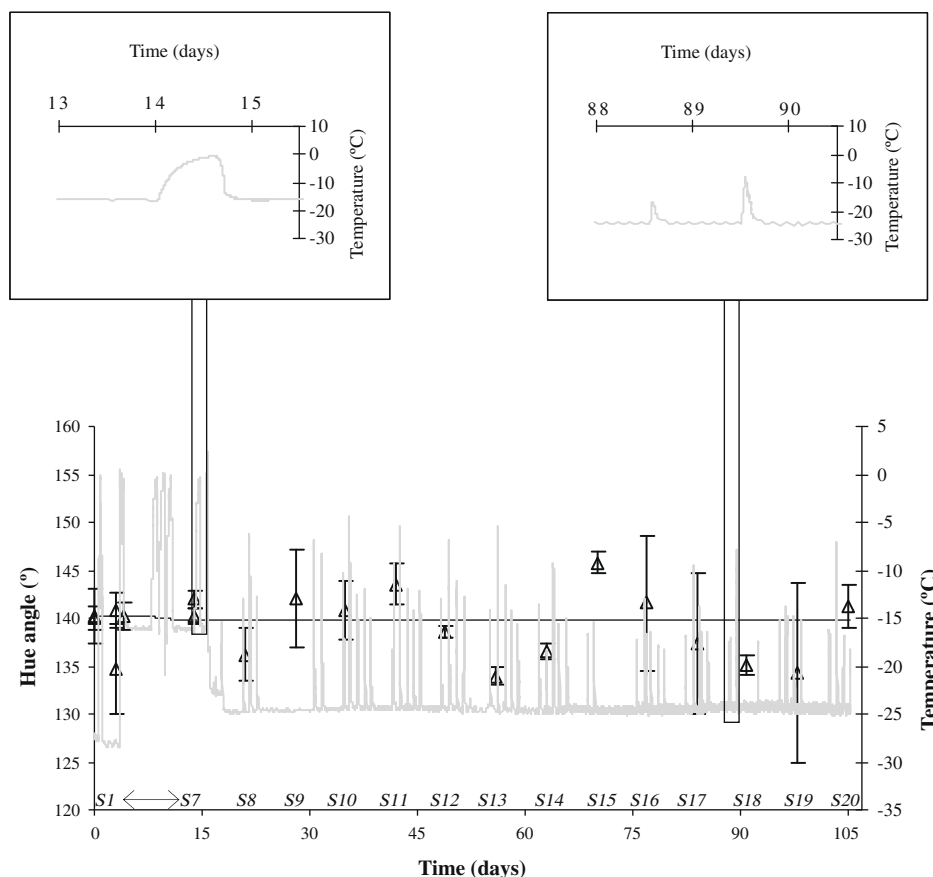


Fig. 7. Watercress hue angle experimental data (Δ) versus zero-order prediction model (—). Temperature abuses profile (—). Bars represent mean \pm standard deviation from three replicates. Small charts represent two specific temperature abuses profiles.

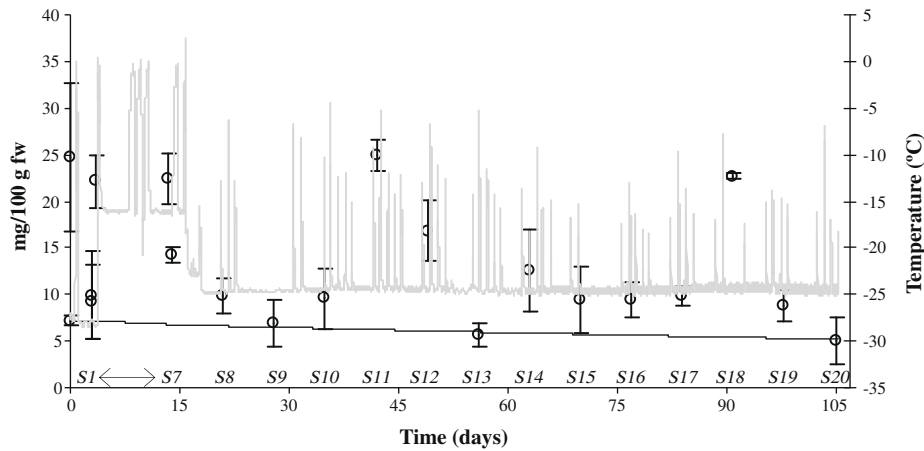


Fig. 8. Watercress ascorbic acid experimental data (○) versus first-order prediction model (—). Temperature abuses profile (—). Bars represent mean \pm standard deviation from three replicates.

Interlaboratory temperature abuse studies on frozen foods, conducted as part of the EU Concerted Action project 'The Preservation of Frozen Food Quality and Safety throughout the Distribution Chain', were reported by Bøgh-Sørensen (1999). The simulations were run in the outermost corner of a pallet of frozen food placed at room temperature, in a display cabinet and during transport of frozen food products to the home. The results indicated small effects on product quality.

The quality of the frozen product, submitted to temperature abuses, is probably dependent, besides other factors previously re-

ferred, on the temperature simulation plan and on the raw material used in the study.

In general, sensory (colour) and nutritional (vitamin C) quality of the frozen watercress was not impaired by the imposed temperature abuses. The reason of this behaviour might have been due to the blanching process applied, which contributed to the stabilisation of the frozen watercress during storage.

3.2. Time temperature profile and quality parameters prediction

In order to compare the experimental effect of temperature abuses on watercress ascorbic acid and hue angle with the prediction models (Eqs. (8) and (9), respectively), the experimental data were plotted against the prediction models values using kinetic parameters determined under isothermal conditions (Table 2).

The hue angle results showed that the prediction model obtained with the isothermal kinetic parameters fitted well the experimental data with the temperature abuses (Fig. 7). In what concerns ascorbic acid, the fluctuation of the experimental content showed higher values than the prediction (Fig. 8). Nevertheless,

Table 3
Published AA kinetic parameters for other vegetables under frozen storage (Giannakourou and Taoukis, 2003).

Kinetic parameters	Green peas	Spinach	Green beans	Okra
E_a (kJ mol ⁻¹)	97.9	112	101.5	105.9
$k_{ref - 20\text{ °C}}$ (d ⁻¹)	0.00213	0.00454	0.00223	0.00105
C_0 (mg 100 g ⁻¹)	28.5	31.1	25.3	28
R^2	0.958	0.992	0.967	0.868
Kinetic model	First-order	First-order	First-order	First-order

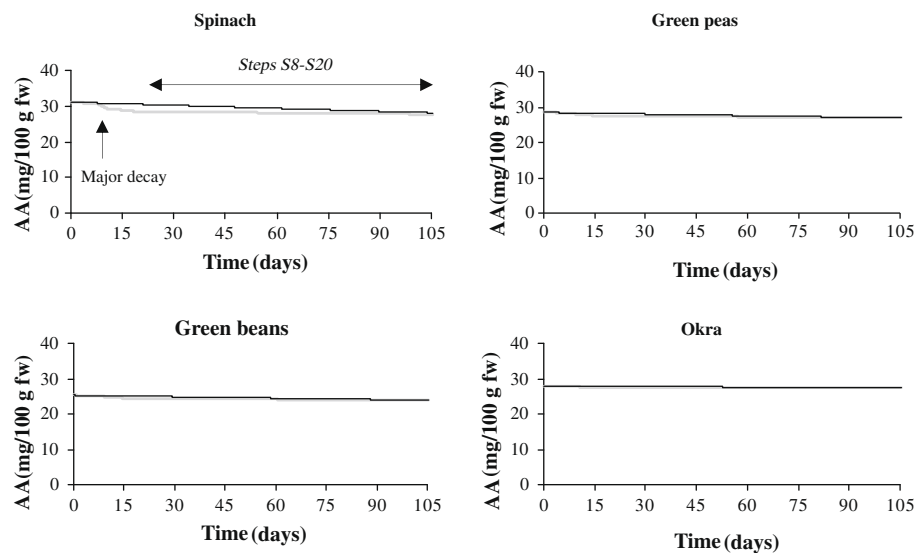


Fig. 9. Spinach, green peas, green beans and okra ascorbic acid prediction models for constant temperature (-18 °C ; black line) and for temperature abuses following the storage plan (grey line).

some experimental points and the final value were in the same range of the predicted ascorbic acid values (about 7 mg 100 g fw⁻¹).

Ascorbic acid profiles for other green vegetables were also simulated using kinetic data from Giannakourou and Taoukis (2003) (Table 3). It was possible to conclude that spinach was predicted more sensitive to the imposed temperature abuses rather than green beans, green peas, okra (Fig. 9) and watercress (Fig. 8). It was also concluded that the first fifteen days of simulation (transportation from factory to store; S1–S6) were crucial for the decay of AA content, since from that point until the end of the plan (customer storage) the AA was decreasing at a lower rate.

These behaviours are related to the properties of each vegetable. The watercress samples are slabs and the heat transfer is completely different from leaves like in the Giannakourou and Taoukis (2003) spinach study, where its surface area is higher and it is not well protected like the watercress slabs. Nevertheless, a good prediction is always dependent on the estimated kinetic parameters under the isothermal studies and its application under different dynamic temperature conditions.

Conclusion

The applied blanching process, although it caused degradation on the vitamin C content and some colour and microstructure changes, might have contributed to the stability of the frozen product during storage. In general, the physical and nutritional studied parameters of the frozen watercress were not impaired by the imposed temperature abuses.

Freezing offers today a large number of advantages demanded by the consumer, e.g. preserving surplus raw material and bridging seasonal gaps in the food supply, as well as convenience, availability, consistent quality and safety.

The main problems affecting the frozen food industry for many years have been related to the storage time and product temperatures, not so much on the basic knowledge of maintaining shelf life, but on handling storage and transport time and temperatures. The quality and acceptance of frozen foods can be further improved if a greater emphasis is laid on avoiding temperature abuses. The need for lower and steady temperatures is fully accepted by everyone involved in the food chain (Eek, 2001).

From an industrial and commercial point of view, the storage stability (with the studied temperature abuses) of frozen watercress slabs represents a valuable advantage for distributors and retailers, and is also a convenient and healthy option to the final consumer.

This kind of study is an important tool, allowing the prediction of the outcome of different storage and distribution conditions, aiming to improve the products quality and cold chain settings.

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