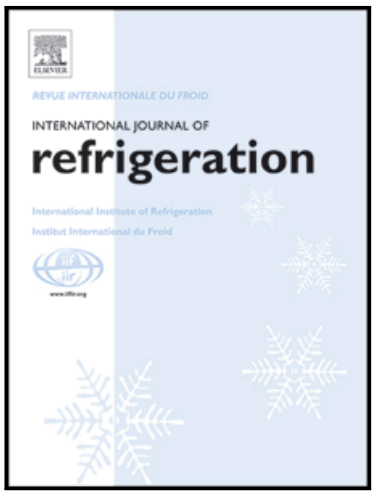

Quality changes kinetics of apple tissue during frozen storage with temperature fluctuations

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Highlights

- Quality changes in apple tissue were studied during frozen storage.
- Temperature fluctuations significantly affect the apple quality during storage.
- The quality data were fitted to first order kinetics.
- A developed kinetic model can be used to predict the apple quality evolution.

Quality changes kinetics of apple tissue during frozen storage with temperature fluctuations

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Abstract

Apple quality after freezing is affected by temperature fluctuations during cold storage. Ice re-crystallization and sublimation occur slowly at a constant temperature over a long period of storage, and more rapidly during fluctuating temperatures. These phenomena impact fruit quality, thus reducing storage life. To this end, apple tissue samples were frozen and subsequently stored in three different freezers set at -12 ± 3 °C, -18 ± 3 °C, and -23 ± 3 °C. In each freezer, three different compartments were created to achieve different amplitudes of temperature fluctuations: (i) low (± 0.1 °C), (ii) medium (± 0.5 °C) and (iii) large (± 1.8 °C). Frost formation, drip loss, color changes and vitamin C (ascorbic acid) content were measured during five months of storage. The results revealed that apple quality was strongly affected by the temperature fluctuations during frozen storage. The kinetic models were calibrated using apple quality data collected at low and large amplitudes of fluctuations. The temperature dependency was successfully incorporated using an Arrhenius equation that integrates the temperature fluctuations. The kinetic models were validated using apple quality data gathered at a medium amplitude of fluctuation. In addition to a kinetic model, a physical model was applied to predict frost formation.

Keywords: Freezing, Cold chain storage, Temperature oscillation, Quality loss, Modeling

1. Introduction

Fruit are perishable plant-based products because of their high-water content. Quality attributes, including appearance, texture, taste, and flavors are important attributes for consumer appreciation and commercial value of fruit. The frozen fruit market has risen considerably (Combris et al., 2007). Fruit processing operations, cold chain storage and distribution affect quality and stability. The freezing process and subsequent storage have been used as a means for preserving fruit quality. However, fruit poses a specific challenge with respect to freezing and storage. The tissue microstructure of fruit is comprised of cells of different sizes and shapes, and interconnected cell walls and intracellular air spaces, also of different sizes and shapes (Herremans et al., 2015; Vicent et al., 2017).

During freezing, ice crystals will be formed throughout cells and interconnected cell walls. Ice re-crystallization (Ullah et al., 2014; Ndoye and Alvarez, 2015) and ice-sublimation (Pham and Mawson, 1997; Campañone et al., 2001) occurring during frozen storage, enhance ice crystals to resize, redistribute and weight loss, thus modify the fruit microstructure and affect fruit quality (Zaritzky, 2000; Ho et al., 2013). These phenomena are influenced by the frozen water stability (Giannakourou and Taoukis, 2003; Taoukis and Giannakourou, 2004) and occur slowly at a constant low temperature over a long period of frozen storage, but more rapidly in fluctuating temperature conditions (Cook and Hartel, 2010; Ullah et al., 2014). Ice crystal growth produces undesirable effects such as cell-damage, leading to cells failing to reabsorb water during thawing, causing the water-soluble nutrients to leach out (Zaritzky, 2000; Gormley et al., 2002; Gonçalves et al., 2011a, 2011b). In case of ice-sublimation, Pham and Willix (1984) and Delgado and Sun (2007) suggested that air temperature, air velocity, relative humidity and water activity create a water vapor pressure gradient that acts as a driving force towards frost formation during storage. Moreover, temperature fluctuations increase the average size of the water vapor pressure gradient, resulting in superficial ice-

sublimation. Ice-sublimation causes weight loss, shrinkage of the tissue structure, and freeze burn (Reid and Perez Albela, 2006) that also result in quality changes.

Several studies have reported the quality loss kinetics during storage at isothermal and/or non-isothermal conditions of frozen greens (Giannakourou and Taoukis, 2003), peas (Serpen et al., 2007), watercress (Cruz et al., 2009; Gonçalves et al., 2009), broccoli (Gonçalves et al., 2011a), pumpkin (Gonçalves et al., 2011b) and spinach (Dermesonluoglu et al., 2015). Bustabad (1999) investigated the effect of storage temperatures (-13 and -18 ^{o}C) on weight loss in beef and pork during six months of storage. The author reported a large amount of weight loss in pork for each storage temperature. In addition, he urged that weight loss during frozen storage was due to ice-sublimation, which is strongly linked to the difference in moisture content between products, the storage temperature and the packaging material. Reid and Perez Albela (2006) studied the impact of storage temperatures on frost forming in frozen storage of starch gel under isothermal and non-isothermal conditions. Laguerre and Flick (2007) reported frost formation in melon and potato due to temperature fluctuations in a domestic freezer. The authors suggested that during frozen storage of foods packed in a non-adhering material, the frost formation occurs due to superficial ice-sublimation and re-freezing on the inside the packing material. Phimolsiripol et al. (2011) pointed out the effect of temperature fluctuations on weight loss in frozen bread dough. On the other hand, few studies have focused on fruit quality during freezing and frozen storage. González et al. (2002) showed a color change in Spanish raspberry during freezing and frozen storage at a constant temperature of -24 ^{o}C. Chassagne-Berces et al. (2010) investigated the impact of freezing rates on two apple cultivars (Golden Delicious and Granny Smith) and mango, and reported the quality changes (such as color, texture and soluble solids).

The quality of frozen fruit depends on numerous factors, including the initial quality, storage temperature conditions and time. If frozen fruit undergoes temperature fluctuations during

frozen storage, ice is subjected to recrystallization and sublimation processes leading to fruit quality changes, and that may override the positive effect of the frozen storage. However, literature contains limited data on fruit quality changes during temperature fluctuation scenarios. Therefore, this paper aims to assess the changes of apple quality attributes during frozen storage at three different temperatures: -12 ± 3 ^{o}C, -18 ± 3 ^{o}C and -23 ± 3 ^{o}C created in three different freezers. The tissue structure of apple fruit with a relatively large amount of air spaces is well suited to investigate this and contribute to our understanding of the effect of temperature fluctuations and how it can be alleviated.

2. Materials and methods

2.1 Apple samples and preparation

'Jonagold' apples (*Malus* × *domestica* Borkh.) were purchased from a local supplier in Paris (France). After receiving 50 kg apples were immediately placed in a room at 4 °C. On the next day, apples were peeled using an apple peeler (Starfrit, Québec, Canada) and slices of approximately 5 g were excised from the apple cortex tissue using apple wedger (Starfrit, Québec, Canada). The excised slices were portioned (to 250 g) and hermetically sealed in polyethylene bags (each with a headspace of approximately 35% of the total volume). In total 55 packed samples were made for freezing and subsequent storage experiments.

2.2 Freezing method

Freezing was carried out by placing packed bags in air blast freezer set at -33 °C. Within the blast freezer, three shelves were created using a wire mesh and on each shelf, the packed samples were loaded. The sample and freezer temperatures were logged throughout freezing using calibrated thermocouples (type T thermocouple of 0.2 mm) attached to a data logger system connected to a computer. For sample temperature records, a thermocouple was inserted into the core of a slice and placed in one of the representative bags. Freezing was carried out in a batch process and for each batch, a representative bag was placed in the

middle position of the freezing chamber. The air freezer temperature was recorded as well. Freezing was accomplished once the slowest cooling point, i.e., sample center in the representative bag, reached -23 ^{o}C. These measurements ensured that the freezing temperature in each bag was reached and were used to estimate the global freezing rate. As a result, a global freezing rate of 1.6 ^{o}C per min. was computed as the mean value of the ratio of the temperature difference between ambient temperature (18 ^{o}C) and the freezing temperature (-23 ^{o}C) divided by the time difference from ambient temperature to freezing temperature. Once frozen, the samples were stored immediately in different storage conditions as detailed in Section 2.3.

2.3 Frozen storage experiments

Frozen samples were instantly distributed in three horizontal freezers (Electrolux, ECM30132W), which had internal dimensions: 113 × 43 × 62 cm each and set at -23 ± 3, -18 ± 3 and -12 ± 3 °C, respectively. In each freezer, three compartments (namely I, II and III) were created to achieve different amplitudes of temperature fluctuations during a five-month of storage as outlined in Fig. 1. Mean temperatures and fluctuations were set using an electronic temperature controller (Eliwell WM961) installed on each freezer as detailed by Ndoye and Alvarez (2015).

Compartment I: the first partition was formed with a polystyrene box of 3 cm thickness (with internal dimensions: 30 × 20 × 30 cm) to represent a very good practice for frozen storage (smallest amplitude of fluctuations). Six bags of frozen samples, each kept in separate cardboard boxes of 2.5 mm thickness (with internal dimensions: 10 × 10 × 10 cm) were placed in this partition (at the same level horizontally as shown in Fig. 1).

Compartment II: the second partition was created with a cardboard box of 3 mm thickness (Rajapack, France with internal dimensions: 40 × 30 × 35 cm) to simulate a medium amplitude of fluctuating temperature, corresponding to a good practice in the cold chain

regime. Six bags of frozen samples, each kept in separate cardboard boxes of 2.5 mm thickness (with internal dimensions: 10 × 10 × 10 cm), were instantly placed in this partition (at the same level horizontally as sketched in Fig. 1).

Compartment III: the last partition was made with no insulation material to mimic a poor practice of fluctuating temperature conditions in frozen storage. Six bags of frozen samples were placed in this compartment (at the same level horizontally as presented in Fig. 1). The combination of three compartments with three temperature scenarios makes nine different storage conditions. These conditions were considered to simulate the temperature scenario that could be encountered in cold chain handling, i.e., during the frozen storage and distribution chain. For each storage condition, air-freezer, air-compartment and core sample temperatures were recorded every five minutes using calibrated type T thermocouples (0.2 mm diameter) connected to a data logger system coupled to a computer. The sample temperature records were made from a single slice placed at the center of the representative bags of frozen tissue samples. The air-compartment temperature was logged in each compartment, and the air-freezer temperature was also monitored as indicated in Fig. 1.

Fig. 2a-b highlights the air time-temperature profile in the three compartments at -12 ± 3 ^{o}C and -23 ± 3 ^{o}C, respectively. Air temperature fluctuations of ± 0.1, ± 0.5 and ± 1.8 ^{o}C were achieved in compartment I, II and III, respectively, and will further be referred to as low, medium and large amplitude of fluctuations throughout this work. It was noticed that the temperature variations were smaller in compartment I due to the insulating effect. These amplitudes of fluctuation values were fairly similar at each storage temperature set in the three freezers as revealed in Fig. 2a-b. Indeed, due to the on/off control operation of the freezing machine, the time cycles ranged between 26 and 34 min for the three scenarios. Fig. 3 shows the product-temperature profiles in three compartments established in the freezer set at -23 ± 3 ^{o}C. Obviously, the variation of the product temperature in compartment III was by

far larger than the one in compartment I and II. This discrepancy was noticed in almost each storage condition. For instance, the product temperature of the apple samples was observed to vary within a range of ± 0.06, ± 0.12 and ± 0.55 ^{o}C for compartment I, II and III, respectively. The fluctuations of the sample center's temperature were virtually equal for each freezer temperature. Monitoring of the sample temperature in each bag throughout the storage experiments was not possible; instead, the sample temperature was recorded for one representative bag for each condition. Nevertheless, each bag packed with samples might experience slightly different temperature regime depending on the position. For that reason, the product-temperature in each compartment was used during modeling as detailed in Section 2.5.2.

2.4 Quality measurements

The first measurements for the quality indices were carried out at 0 d for drip loss, color changes and vitamin C (ascorbic acid) content. The next analyses also, included frost formation and were undertaken after 15 d and then monthly during storage at different conditions over a 155 d storage period. No weight loss was observed during frozen storage for all different conditions. For each storage condition, one sample bag was randomly selected for quality measurements, from which four replicates of approximately 50 g were made, using an analytical balance (Sartorius GMBH, Göttingen, German), capable of weighing a maximum mass of 424 g with a precision of 0.001 g. Frost formation was measured in four replicates, followed by drip loss using the first three replicates. The fourth replicate was used for measuring color changes and vitamin C (ascorbic acid).

2.4.1 Frost formation

Frost formation in frozen apple tissue was evaluated by placing the weighted frozen samples in a wire-mesh basket and shaking them gently over a weighted absorbent paper. Then the entire frost layer on the absorbent paper was carefully collected. From the preliminary tests, it

appeared that shaking during 30 s was sufficient to extract the frost formed on the surface of the apple slices. Frost formation (%) was determined by weighing the collected ice on absorbent paper and dividing by the mass of the frozen samples. An analytical balance (Sartorius GMBH, Göttingen, German) was used. For each storage condition, four replicates were used for frost analysis. All analyses were performed in cold store and were completed in less than 2 min.

2.4.2 Drip loss

Analysis of drip loss in frozen apple tissue was performed using the first three replicate samples after frost measurement. Each weighed sample was placed over a weighed dry absorbent paper kept in a separate plastic container (10 × 10 × 7 cm internal dimensions) and sealed to prevent moisture loss during thawing. The samples were allowed to thaw in a fridge set to 4 ^{o}C overnight as detailed by Agnelli and Mascheroni (2002). From preliminary test, this was sufficient to attain the same temperature value of 4 ^{o}C for all the samples irrespective of the different storage conditions. Drip loss was then quantified at each time-point by weighing the absorbent paper and expressed as a percentage. For each storage condition, three replicates were used for drip loss analysis.

$$\text{Drip loss} = \frac{m_{wp} - m_p}{m_s} \times 100\ \% \quad (1)$$

where m_s, m_{wp} and m_p are the mass (g) of the frozen apple tissue, wet absorbent paper after thawing and dry absorbent paper, respectively.

2.4.3 Color measurement

The color change of frozen apple tissue was imaged using a digital camera (Panasonic DMC-TZ57, Osaka, Japan). To have the same calibrated dimensions in all images and isolated the tissue from the background, a polystyrene box was designed to provide a fixed distance between the camera and samples. Furthermore, a hole was made at the top of a box from

which the photographs were captured. Digital images of 1920 × 1080 pixels were taken at magnification ×20. Image processing of color changes was performed using Image J 1.48v (National Institutes of Health, USA). The brown surface area of the apple tissue slices was segmented using the hue-saturation-brightness (HSB) color space. A color change was then quantified as the ratio of the brown surface area to the total surface area of the apple tissue slices expressed as percentage. For each storage condition, the color was analyzed for three replicates of three slices.

2.4.4 Vitamin C content

Vitamin C (ascorbic acid) content in frozen apple tissue was determined by titration (AOAC, 1984) using a blue dye solution of 2,6-dichloroindophenolindophenol (6 %). 50 g of sample for each storage condition was allowed to thaw in a dark room. The thawed sample was then homogenized with 50 g of metaphosphotic acid (3 %) using a pestle and mortar and filtered through Whatman n^{o} 42 filter paper. From the filtered extract, 1 g of juice was weighed in 250 mL volumetric flask and diluted to 50 g with distilled water. An analytical balance (Sartorius GMBH, Göttingen, German) was used. The mix solution was titrated with 6 % of 2,6-dichloroindophenolindophenol solution until a pink color was observed. Prior to vitamin C (ascorbic acid) determination in apple tissue, the titration method was optimized using standard solutions of ascorbic acid, which were prepared and titrated with blue dye (data not shown). Vitamin C (ascorbic acid) determination was performed in three replicates per storage condition and then vitamin C (ascorbic acid) content was quantified in g ascorbic acid per 100 kg of apple tissue as follows:

$$[\text{Vitamin C}] = \frac{VF}{m} \times 100 \quad (2)$$

where V is the volume of 2,6-dichloroindophenolindophenol consumed (mL), F is a conversion factor of the ascorbic acid consumed with blue dye (g L^{-1}), and m is the mass of the juice sample (g).

2.5 Modeling

2.5.1 Kinetic model for quality indices

Quality changes during frozen storage have been modeled using zero-order and/or first-order kinetics (Giannakourou and Taoukis, 2003; Gonçalves et al., 2011a, 2011b; Phimolsiripol et al., 2011; Dermesonluoglu et al., 2015). The quality changes during storage may be fast or slow depending on the storage temperature conditions and time. The quality changes in frozen apple tissue were described by zero and first-order kinetic models, Eqs. (3) and (4), respectively, under the assumption that quality changes are only dependent on storage temperature, fluctuations and duration. A zero-order kinetic model, Eq. (3) was used to describe color changes, and a first-order kinetic model, Eq. (4) was applied for frost formation, drip loss and loss in vitamin C (ascorbic acid) content during frozen storage at temperature fluctuations.

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = -k_{i,0} \tag{3}$$

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = -k_{i,1}Q \tag{4}$$

where Q is the measured value for each quality attribute, k_i (% d^{-1} or d^{-1}) is the rate constant (the subscripts 0 and 1 correspond to zero and first-order kinetics, respectively) and t (d) is the storage time.

2.5.2 The relationship between kinetic rate and storage temperature

Effect of storage temperature on quality changes kinetic rate was incorporated into the model and estimated using the Arrhenius equation, Eq. (5).

$$k_i = k_{\mathrm{ref,i}} \exp\left(-\frac{E_{a,i}}{R}\left(\frac{1}{T(t)} - \frac{1}{T_{\mathrm{ref}}}\right)\right) \tag{5}$$

where k_i (% d^{-1} or d^{-1}) and $E_{a,i}$ (J mol^{-1}) are the kinetic rate constants and the activation energies, respectively for each quality change, $k_{\mathrm{ref,i}}$ is the rate constant to the respective

quality indicator at a reference temperature, T_{ref} (255 K), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T (K) is the storage temperature varies as a function of time t.

The storage temperatures in Eq. (5) are the product temperatures recorded every five minutes over a period of five months for each storage condition. For kinetic modeling purposes, these temperatures were considered to determine the impact of temperature fluctuation scenarios on quality changes during five months of storage. Therefore, Eq. (5) is valid in the range of temperature fluctuation scenarios studied.

2.5.3 Parameter estimation

Calibration of the models was carried out by fitting the kinetic models to a subset of the data, i.e., data measured at -23, -18 and -12 ^{o}C all with low and large amplitudes of temperature fluctuations. The kinetic model parameters (initial quality value Q_0, rate constant $k_{ref,i}$ and activation energy $E_{a,i}$) were estimated from the calibration dataset for each quality attribute. Realistic values from literature were assigned to these model parameters to initialize the parameter estimation procedure. Gonçalves et al. (2011a) stated the rate constants (d^{-1}) equal to 0.043 for drip loss, 0.0044 for color changes and 0.0068 for vitamin C (ascorbic acid) loss in broccoli. Authors also reported the activation energies (kJ mol^{-1}) equal to 42.3 for drip loss, 53.6 for color degradation and 60.2 for vitamin C (ascorbic acid) loss. Phimolsiripol et al. (2011) showed estimated rate constant of 3.3 d^{-1} and activation energy of 72.4 kJ mol^{-1} for ice sublimation in bread dough. Model implementation, calibration and validation were done using OptiPa, a dedicated software to estimate parameters of ordinary differential-based equations (ode) using a least squares method (Hertog et al., 2007). The model equations were numerically solved using an ode45 solver.

The kinetic models were validated using the measured dataset gathered at -23, -18 and -12 ^{o}C all with medium amplitude of fluctuation. Validation of the models was done to predict the quality changes during five months of storage. The mean absolute percentage error (MAPE)

was analyzed for each quality indicator to evaluate the accuracy of the model prediction between the predicted and measured values as shown in Eq. (6).

$$\mathrm{MAPE} = \frac{100}{\mathrm{n}} \sum \left| \frac{x_{\mathrm{obs}} - x_{\mathrm{pre}}}{x_{\mathrm{obs}}} \right| \tag{6}$$

where n is the number of observations, x_{obs} is the observed value for each time-point, and x_{pre} is the predicted output for that observation.

2.5.4 Physical model for frost formation

In addition to the kinetic approach applied to describe frost formation in apple tissue during storage. A physical model was also implemented to predict frost formation kinetics as a result of ice sublimation. In principle, ice-sublimation depends on the water vapor pressure difference between the product surface and the surrounding air as suggested in the literature (Pham and Mawson, 1997; Pham and Willix, 1984; Campañone et al., 2005; Phimolsiripol et al., 2011). For that reason, the simplest physical model that incorporates water loss from the product leading to frost formation is due to vapor pressure gradients proposed as in Eq. (7).

$$\frac{\mathrm{d}F_m}{\mathrm{d}t} = k_m A \left(a_w \rho_s - \frac{RH}{100} \rho_a \right) \tag{7}$$

with $\rho = \dfrac{M_r P(T)}{RT}$

where F_m is the frost formation (kg), k_m is the mass transfer coefficient between air and product (m s^{-1}), A is the surface area of the sample (m^2), a_w is the water activity, RH is the air relative humidity (%), ρ_s and ρ_a are the saturation water vapor concentrations (kg m^{-3}) at the product surface (T_s) and the surrounding air (T_a), respectively, P is the saturation vapor pressure at temperature T, M_r is the molecular mass of water (kg mol^{-1}), R is the universal gas constant (J mol^{-1} K^{-1}) and t is the storage time (s).

The physical model parameters $k_m \times A$ (m^3 s^{-1}) and initial frost formation ($F_{m,o}$) were estimated using OptiPa. a_w and RH were assumed and kept fixed for all experimental

conditions. Therefore, this physical model describes that the rate of frost formation is driven by the water vapor pressure gradient between the product surface and the surrounding air, which depends on temperature. The model uses only positive rate of frost formation.

2.6 Data analysis

The resulting quality data were imported into Matlab (R2015a, Mathworks Inc., Natick, MA, U.S.A), where the statistical analysis (factorial ANOVA with replication) was performed to assess the significant effects of the storage time-temperatures at different fluctuations on the quality indices of frozen apple tissue. The Tukey Kramer test for multiple range comparisons ($p < 0.05$) was applied to identify the difference among the measured mean values expressed as mean and standard deviation ($\bar{x} \pm$ S.D.) for each quality parameter.

3. Results and discussion

3.1 Frost formation

Fig. 4 shows the measured frost formation data, together with the corresponding fitted values in apple tissue stored during five months at -23, -18, and -12 °C all with low and large amplitudes of temperature fluctuations. The results show good agreement between the measured values and fitted data. The rate of frost formation increased significantly ($p < 0.05$) during five months of storage in all conditions. At a large amplitude of fluctuation, the cumulative frost formation was 9.8 %, 11.2 % and 13.1 % at -23, -18 and -12 ^{o}C, respectively over a five-month period of storage. At low amplitude of fluctuation, the frost formation was 1.9 %, 2.1 % and 4.4 % in the same period at -23, -18 and -12 ^{o}C, respectively. These results show that frost formation increased with increasing storage temperature and amplitude of fluctuation. It appears that for controlling frost formation, it is more important to reduce temperature fluctuations than to reduce mean storage temperature.

Pham and Willix (1984) and Delgado and Sun (2007) mentioned that the difference between the water activity of the product and relative humidity of the surrounding air creates a vapor

pressure gradient that is the driving force for ice-sublimation. In our experiments, the temperature fluctuations tend to increase the average magnitude of the water vapor pressure gradient, which induces irreversible moisture migration from the interior of the samples towards the surface and later to the air as the package follows the cooling-warming cycles faster than the product samples. As discussed in Section 2.4, no weight loss in the packed samples was observed throughout storage. This implies that the ice-redistribution was only between the frozen apple samples and the air in the packaged bags. Consequently, frost formation leads to weight loss and produces a desiccated surface in frozen samples that can probably change the product microstructure. These findings are consistent with the results of Phimolsiripol et al. (2011) in frozen bread dough during storage at isothermal and non-isothermal temperatures, in which weight loss (frost formation) reported to increase with temperatures and increased more at the fluctuating scenario. Laguerre and Flick (2007) showed the effects of the temperature variations on frost formation in melon and potato balls stored in a domestic freezer for three-month duration. As such, a large amount of frost equal to 5.2 % was stated in melon compared to 1.8 % in potato balls at the end of the storage period at -25 ± 8.5 ^{o}C. Differences in the frost formation were probably due to differences in moisture content between the two products used (melon and potato) and ours: 92 % for melon and 78 % for potato as compared to a moisture content of 85.8 % of apple tissue (Vicent et al., 2017). Moreover, frost fractions in melon and potato differ from the current work and may be explained by the differences in temperature fluctuating conditions that were applied between the two studies, as well as differences in water activity between the products.

3.2 Drip loss

Fig. 5 represents the effect of storage temperature and amplitude of fluctuation on drip loss during five-month storage. A satisfactory fit was obtained between the measured data and fitted output of drip loss during storage at both low and large amplitudes of temperature

fluctuations. Drip loss was 6.56 % at 0 d as shown in Fig. 5. The results revealed that drip loss increased significantly ($p < 0.05$) with mean storage temperature and amplitude of fluctuating temperatures during the storage period (Fig. 5). The drip loss of samples stored during five months at -23 ^{o}C and low amplitude of fluctuation was 17.8 % and was smaller than that at large amplitudes of temperature fluctuation (21.9 %). These findings demonstrate that drip loss in apple tissue becomes more evident as the storage temperature increases with time (Fig. 5). For this reason, a high amount of drip loss equal to 20.3 % and 25.7 % was measured after five months of storage at -12 ^{o}C with low and large amplitudes of fluctuation, respectively. However, it was not possible to reduce drip loss below 15 % for the condition studied, indicating that the freezing process may also contribute significantly to drip loss.

These results can be explained by the growth of ice crystals during frozen storage due to temperature abuse (Ndoye and Alvarez, 2015). Ice resizing and redistribution can disrupt cell walls of the fruit and lead to loss of cell turgor and leaching of cell contents (especially water-soluble nutrients) during thawing (Gonçalves et al., 2011a). Several researchers pointed out that ice re-crystallization occurs slowly at the constant temperatures over a long period of storage, but more rapidly during temperature fluctuations that influence drip loss during thawing. The drip loss results presented in this work are in agreement with those reported in frozen broccoli by Gonçalves et al. (2011a). The authors found a considerable increase in thawing drip loss with temperature of 12 %, 43 % and 87 % after 121 d of storage at isothermal temperatures of -25, -15 and -7 ^{o}C, respectively. A substantial drip loss change of approximately 143.5 % reported in broccoli after 57 d of storage at non-isothermal temperature condition. Similarly, Gormley et al. (2002) showed that thawing drip loss in broccoli was affected more at non-isothermal temperatures as compared to that at the isothermal temperature scenario during eight months of storage.

3.3 Color changes in frozen apple

Fig. 6 shows the combined experimental and fitted data for color changes in apple tissue stored at -12, -18 and -23 °C all at low and large amplitudes of fluctuation. A close description between the measured and fitted values of color changes is observed, except for apple tissue stored at -12 ^{o}C with low amplitude of fluctuation. The results suggest that the color changes more rapidly ($p < 0.05$) with increasing mean storage temperature and amplitude of fluctuation. For instance, almost 69.5 % of the surface area of the apple tissue appeared brown after five months of storage at -12 ^{o}C with a large amplitude of fluctuation, whereas apple samples stored at -18 and -23 ^{o}C both with a large amplitude of fluctuation showed color changes of approximately 35.2 % and 7.9 % respectively. In apple samples stored for five months at -12, -18 and -23 ^{o}C and low amplitude of temperature, only 22.8 %, 10.0 % and 1.7 % of the sample surface areas appeared brown. These results demonstrate that color remained almost unchanged for the apple samples stored at -23 ^{o}C with low amplitude of fluctuation throughout the storage period. On the contrary, the apple samples stored at -23 ^{o}C with large amplitude of temperature fluctuation showed a color change of 8.0 %, meaning that reducing the mean temperature may be more important than reducing fluctuations with respect to color stability. Color change appeared to be the most sensitive quality indicator during frozen storage of apple slices among those presented in this work. The color evolution observed during frozen storage is likely a consequence of the enzymatic polymerization of phenolic compounds resulting in brown pigments. Chassagne-Berces et al. (2010) investigated color changes in apples and mango submitted to the different freezing rates, and reported that the freezing process influences more the color changes in apples as compared to mango fruit. The authors suggested that this could be explained by the presence of polyphenols in apples that are more susceptible to enzymatic browning process compared to the carotenoids in mango. In frozen storage, González et al. (2002) investigated color change in Spanish raspberry during 12 months of storage at -24 ^{o}C and found that color change

becomes more distinct after six months of storage. Gonçalves et al. (2011a) reported the influence of storage temperatures on color change of green broccoli and found that broccoli samples stored at high constant temperature (-7 ^{o}C) de-greened faster than broccoli samples stored at -15 and -25 ^{o}C. Furthermore, the de-greening process was most prominent during non-isothermal conditions.

3.4 Loss in vitamin C content

Fig. 7 shows the effects of storage temperature on vitamin C (ascorbic acid) content measured, together with the corresponding model fitted values during five months storage at -23, -18, and -12 °C with low and large amplitudes of temperature fluctuation. A close match was achieved between the measured and fitted values of vitamin C (ascorbic acid) in apple samples, except during the last two months of storage at low amplitude of fluctuation. This is because vitamin C (ascorbic acid) changes in apple tissue were not significant during that period for the three mean temperatures. Vitamin C (ascorbic acid) content was measured immediately after freezing i.e., at 0 d and found equal to 73.5 ± 2.9 mg in kg of apple samples. The results show that vitamin C (ascorbic acid) content differed from that reported by Planchon et al. (2004) in fresh Jonagold apples (119 ± 5 mg in kg of fresh apples). This discrepancy in vitamin C (ascorbic acid) content may be due to effect of freezing on vitamin C (ascorbic acid) content as stated by Tosun and Yücecan (2008) in some fruit and vegetables, as a result of no pretreatment. The authors stated that the freezing process causes vitamin C (ascorbic acid) losses of approximately 22 % to 57 % depending on the product: for example, in okra (45.7 %), potatoes (56.8 %), green beans (38.2 %), broccoli (21.9 %), spinach (36.4 %) and peas (29.1%). During storage, the vitamin C (ascorbic acid) content of apple tissue showed a significantly decreasing pattern ($p < 0.05$) with increasing mean storage temperature and amplitude of fluctuation. At -12 ^{o}C with a large amplitude of fluctuation, the vitamin C (ascorbic acid) decreased faster (68.8 %) after five months of storage. Apple

samples stored at -18 and -23 ^{o}C had vitamin C (ascorbic acid) losses of approximately 62.7 % and 43.5 %, respectively. Moreover, apple samples stored during five months at -12, -18 and -23 ^{o}C with low amplitude of fluctuation had a slower rate of vitamin C (ascorbic acid) loss of 56.2 %, 43.4 % and 29.3 %, respectively. No significant difference in vitamin C (ascorbic acid) content was found in apple samples stored at -23 ^{o}C at low and large amplitudes of fluctuation during the last two months of storage. This trend was also seen for apple samples stored at -18 with low amplitude of fluctuation.

These results agree well with numerous studies that reported a decrease of vitamin C (ascorbic acid) content in some vegetables during frozen storage, especially under temperature abuse (Giannakourou and Taoukis, 2003; Gonçalves et al., 2011a; Gonçalves et al., 2011b; Serpen et al., 2007). These authors reported that loss of vitamin C (ascorbic acid) was mainly due to the irreversible oxidation mechanism. However, it should also be pointed out that part of the vitamin C (ascorbic acid) content may end up in drip loss, since it is a water-soluble compound. In contrast to the results presented here, Gormley et al. (2002) and Cruz et al. (2009) reported that vitamin C (ascorbic acid) content was retained well in strawberries and watercress during storage under temperature fluctuations.

3.5 Kinetic modeling

Frost formation, drip loss and loss in vitamin C (ascorbic acid) content were described well by an Arrhenius first order kinetic model (Figs. 4, 5 and 7 respectively). Experimental results for color changes in apple tissue slices were adequately described by an Arrhenius zero-order kinetic model (Fig. 6). Table 1 shows the kinetic model parameter estimates, together with the standard deviations. Estimated initial quality indices (Q_0) were found for frost mass (0.27 %), drip loss (7.67 %), color change (0.44 %) and vitamin C (ascorbic acid) content (71.8 mg kg^{-1}). The quality kinetic rate constants ($k_{ref,i}$) were 0.017, 0.068 and 0.0059 d^{-1} for frost formation, drip loss and vitamin C (ascorbic acid), respectively, and 0.0088 % d^{-1} for color

changes. The activation energies (E_a) were 14.0, 11.7, 22.5 and 25.3 kJ mol^{-1} for frost formation, drip loss, color changes and loss in vitamin C (ascorbic acid) content, respectively. Estimated parameters are given for a reference temperature of -18 ^oC. The small standard deviations of the estimated model parameters suggest that the kinetic models gave an adequate description of the quality changes during five months of storage at different temperature fluctuations (Figs. 4 - 7). The fitted results clearly show how apple quality attributes change with increasing temperature and/or amplitude of the fluctuation. The kinetic models for drip loss, color changes and vitamin C (ascorbic acid) content were validated using a dataset collected at medium amplitude of temperature fluctuation.

Gonçalves et al. (2011a) stated large estimate of the activation energy (E_a) values (kJ mol^{-1}) equal to 53.6 for color, 60.2 for vitamin C (ascorbic acid) and 42.3 for drip loss in broccoli stored at isothermal temperatures. Smaller E_a values (J mol^{-1}) of 1.7 for color, 3.9 for vitamin C (ascorbic acid) and 4.7 for drip loss were reported for non-isothermal temperatures. Giannakourou and Taoukis (2003) worked with frozen greens during storage at varying time-temperature conditions and found higher values of E_a (kJ mol^{-1}) for vitamin C (ascorbic acid) ranged from 98 to 112. However, no other data on the quality kinetics in frozen apple tissue during storage at fluctuating temperatures were found in the literature. The differences between the kinetic model parameters reported and ours may be due to differences in storage temperature plans that were applied between these studies. In addition, the different food materials used may have different quality changes during frozen storage due to their inherent variabilities.

Fig. 8a-d shows the predicted and measured data together with the mean absolute error for each quality attribute during five months of storage at -23, -18 and -12 ^oC all with medium amplitude of fluctuation. The model prediction accuracy was evaluated by computing the MAPE, which measures the mean absolute error between the predicted and observed values.

The model predicted well drip loss, color changes and loss in vitamin C (ascorbic acid) content with relatively small absolute errors as shown in Fig. 8b-d. However, the predictions of frost formation at -12 and -18 ^{o}C showed large absolute errors (Fig. 8a). The large percentage errors in the predicted frost formation are likely due to the effect of temperature differences between the apple tissue and the surrounding air, water activity and air relative humidity, which were not accounted for by the kinetic model. These results suggest that a physical model is required to describe frost formation.

3.6 Physical model for frost formation

A physical model was applied to describe frost formation due to a vapor pressure difference between the product surface and the surrounding air. Calibration of the model was carried out by fitting the physical model to a data subset collected at low and large amplitudes of fluctuations. Fig. 9 shows the model fitting of frost formation data at low and large amplitudes of fluctuations, respectively. The physical model clearly shows that frost formation increases with increasing storage temperature, amplitude of temperature fluctuations and storage time as discussed in Section 3.1. A better fit was obtained at -12 ^{o}C for both amplitudes of fluctuations. In addition, for apple tissue samples stored at -18 ^{o}C with low amplitude of fluctuation, the model described well frost formation (Fig. 9). On the contrary, the model fit for tissue samples stored at -23 ^{o}C with both low and large amplitude of fluctuations was less good, as well as for samples stored at -18 ^{o}C with large amplitude of fluctuation. The overall fitted physical model parameters $k_m \times A$ and $F_{m,o}$ were 1.77 ± 0.15 m^3 s^{-1} and 0.16 ± 0.8 %, respectively. The model also fails to predict the observed increasing rate in frost formation with time; rather it predicts a linear trend. Fig. 10 shows the prediction of frost formation for the validation dataset collected at a medium amplitude of temperature fluctuation. The MAPE that shows the difference between the measured and predicted values was relatively small at -12 ^{o}C. The physical model thus predicted well frost formation in apple tissue stored at a

higher temperature (-12 ^{o}C) during five months of storage. The large MAPE in the predicted frost formation at -23 and -18 ^{o}C might be related to the diffusion of water vapor into airspaces of the frost layer that may enhance the mechanism of frost formation. Indeed, water vapor transferred into frost layer increases both thickness and density of frost. Armengol et al. (2016) reported that frost layer thickness growth occurs at the beginning of frosting and followed by densification. Lee et al. (2003) also showed that approximately 70 % of the mass flux trasferred into the frost layer thus increases the frost thickness. The densification process of the frost layer is mainly due to diffusion of water vapor in airspace of the frost layer, leading to the increasing rate of frost at lower temperatures. This could describe the frost formation observed in apple samples stored at -23 and -18 ^{o}C at the end of the storage period. Other possible reasons that could explain an inadequate description of frost formation are moisture and temperature gradients within the sample. Furthermore, water activity and air relative humidity were assumed to be constant for all experimental conditions.

If the frozen apple tissue undergoes temperature fluctuations during storage, the fluctuating temperature scenario creates cool-warming cycles between the product surface and the surrounding air. As such, there will be alternating periods in which apple tissue surface temperature is higher than the surrounding air, resulting in ice-sublimation and vice versa. However, Pham and Willix (1984) and Pham and Mawson (1997) remarked that there will be a net ice migration from product to the surrounding airspace as the rate of ice-sublimation, i.e., from the product to the surrounding air space is faster than reverse ice-sublimation, i.e., from the surrounding air space back to the product. This could be explained by the difference in active phase when the ice sublimes from the product to the airspace relative to when the ice sublimes back from the packaging material. Certainly, water vapor pressure differences enhance the mechanism of frost formation that was noted to increase with increasing temperature fluctuations. The model proposed in this work suggests that the mean storage

temperatures and amplitude of fluctuations need to be reduced to control frost formation during cold chain storage of fruit.

The two models utilized in this work for frost formation predictions can be compared in Figs. 8a and 10. Both physical and Arrhenius models showed a partial validity between the predicted and measured data. The two models showed a good prediction of frost at a higher temperature (-12 ^{o}C), but under-estimated the rate of frost formation at lower temperatures (-23 and -18 ^{o}C), as shown in Figs. 8a and 10. This is because the physical phenomena involved in frost formation could not simply be described by temperatures as expressed in the Arrhenius kinetic model. Integrating the water vapor gradient as a driving force for frost formation was also insufficient as shown by the physical model. Change of the frost layer density with time, temperature and moisture gradients within the product, water activity and air relative humidity that change with storage time need to be incorporated in future models.

The frozen fruit industry usually faced with fruit quality alteration during storage and distribution. In case the frozen fruit undergoes temperature abuse during cold storage, the ice-recrystallization and ice-sublimation phenomena can occur that affect the quality attributes, including sensory and nutritional properties (Giannakourou and Taoukis, 2003; Gonçalves et al., 2011a, 2011b; Phimolsiripol et al., 2011; Dermesonluoglu et al., 2015). To reduce quality losses, knowledge on how the fruit quality is affected by temperature fluctuations during frozen storage is required.

4. Conclusions

Quality changes (frost formation, drip loss, color and vitamin C (ascorbic acid) content) in apple cortex tissue were studied during frozen storage under fluctuating temperature conditions. The results demonstrated that quality is affected by storage temperature, fluctuations and duration. High temperatures and/or large temperature fluctuations produce cumulative adverse effects on fruit quality over a long period of storage. This implies that a

controlled cold chain is of the foremost important to manage the apple quality and prolongs storage life.

The change of quality attributes of frozen apple tissue samples in time were described well with zero and first-order kinetic models. The physical model is an alternative approach to describe frost formation based on water vapor differences. The model predictions suggest that other factors may have impacts on frost formation and should be accounted in a more complete model. The results and proposed kinetic and physical models provide insights into the cold chain industry to manage fruit quality during storage and distribution chain by understanding the effect of fluctuating temperatures.

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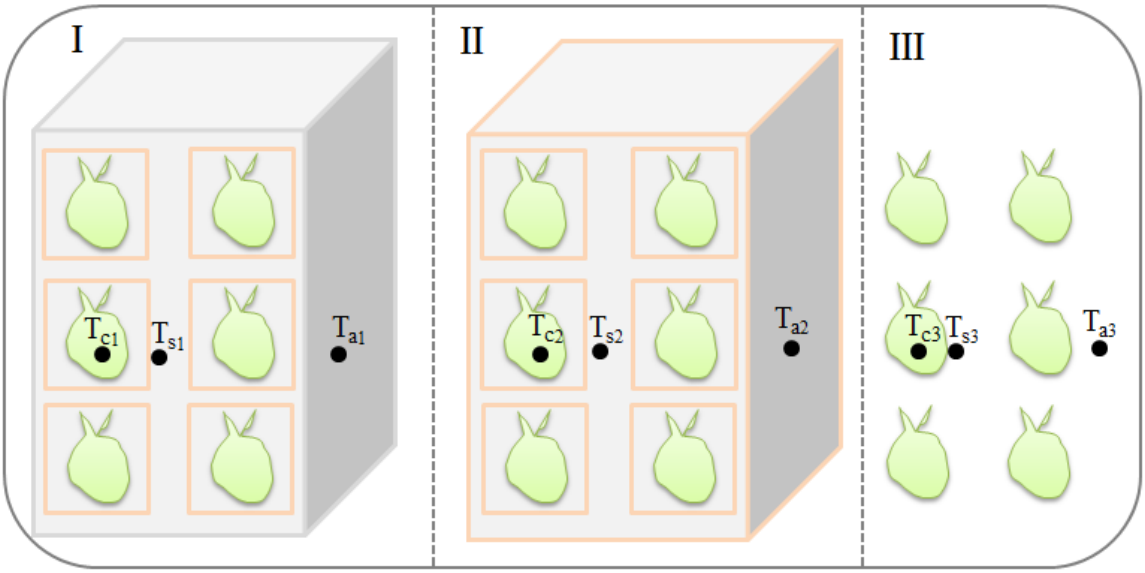

Fig. 1. A simplified scheme of the three partitions namely compartment I, II and III made in each freezer for storage experiments of the apple cortex tissue, designed to show the temperature measuring positions at the apple center (T_c), air-compartment (T_s) and freezer temperatures (T_a).

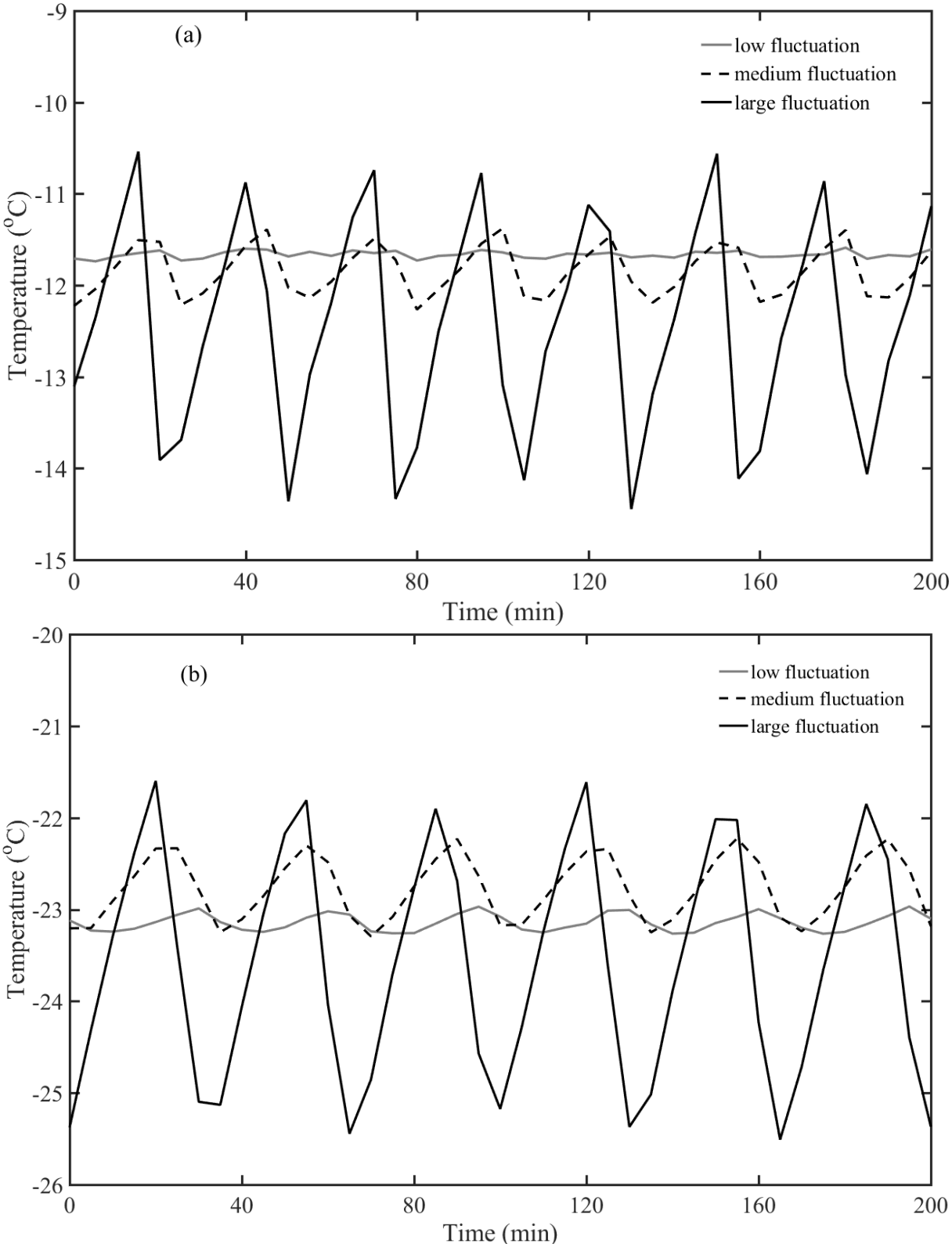

Fig. 2. Illustrations of the air time-temperature profiles in three compartments designed for storage experiments of frozen apple tissue in the two freezers (**a**): set at -12 ± 3 °C and (**b**): set at -23 ± 3 °C.

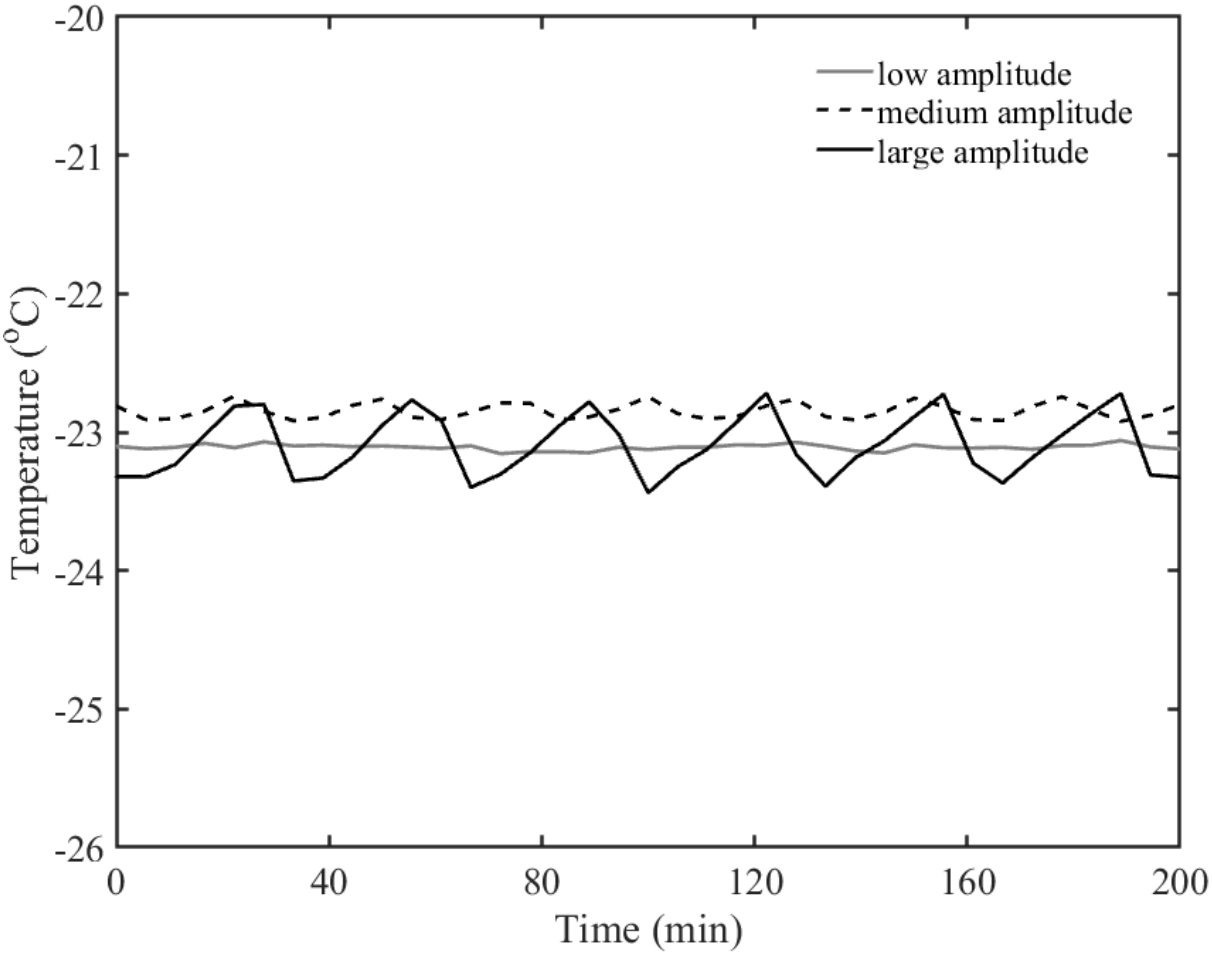

Fig. 3. Graphics of the product-temperature profiles in three compartments created for storage experiments of frozen apple tissue stored in a freezer set at -23 ± 3 °C.

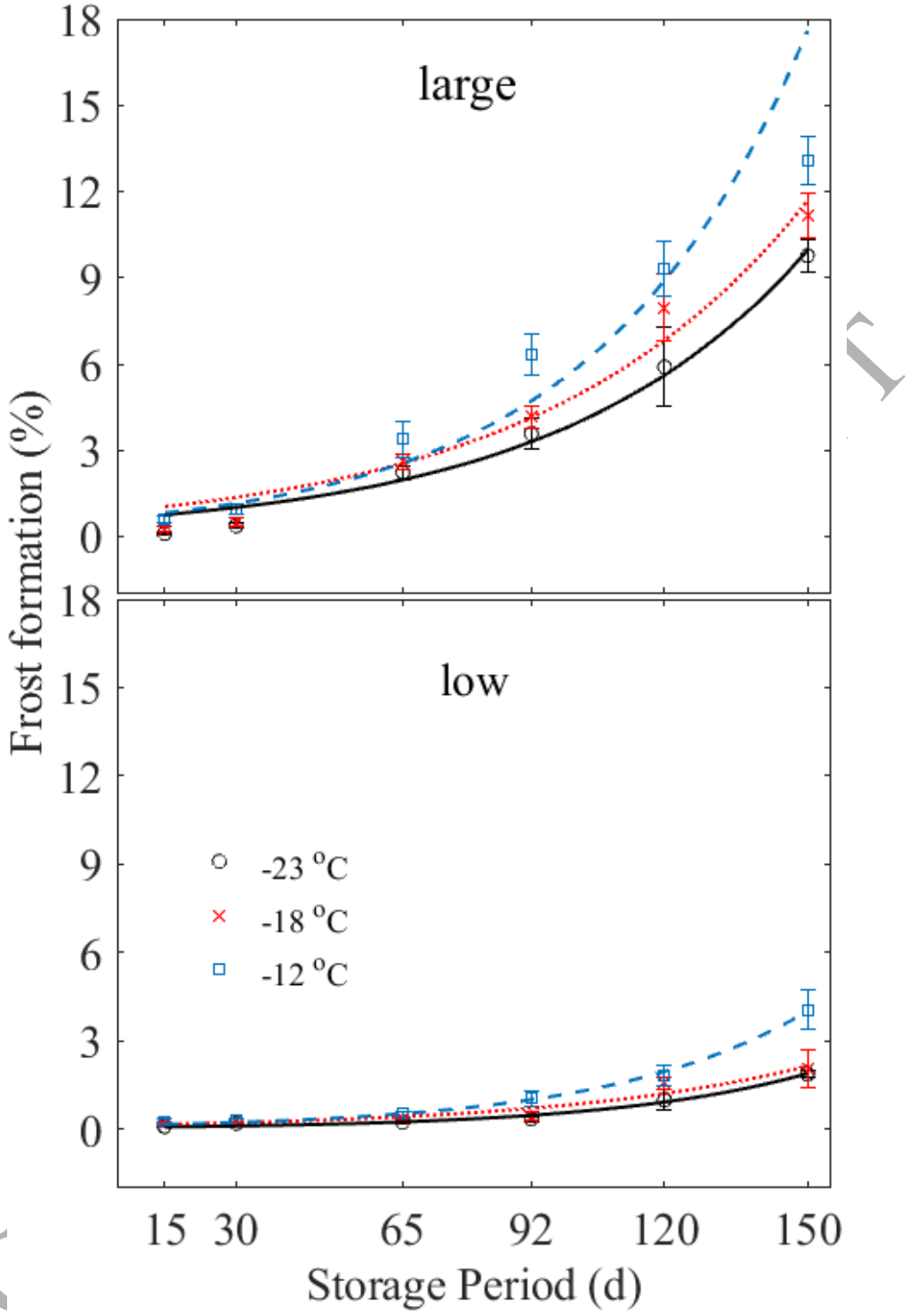

Fig. 4. Effect of mean temperatures with low and large temperature fluctuations on cumulative frost formation (%) of the frozen apple tissue during frozen storage under fluctuating temperature regimes. The lines are the fitted data for frost formation. The points are the means of the measured values from four replicates, with error bars representing the standard error at each time-point.

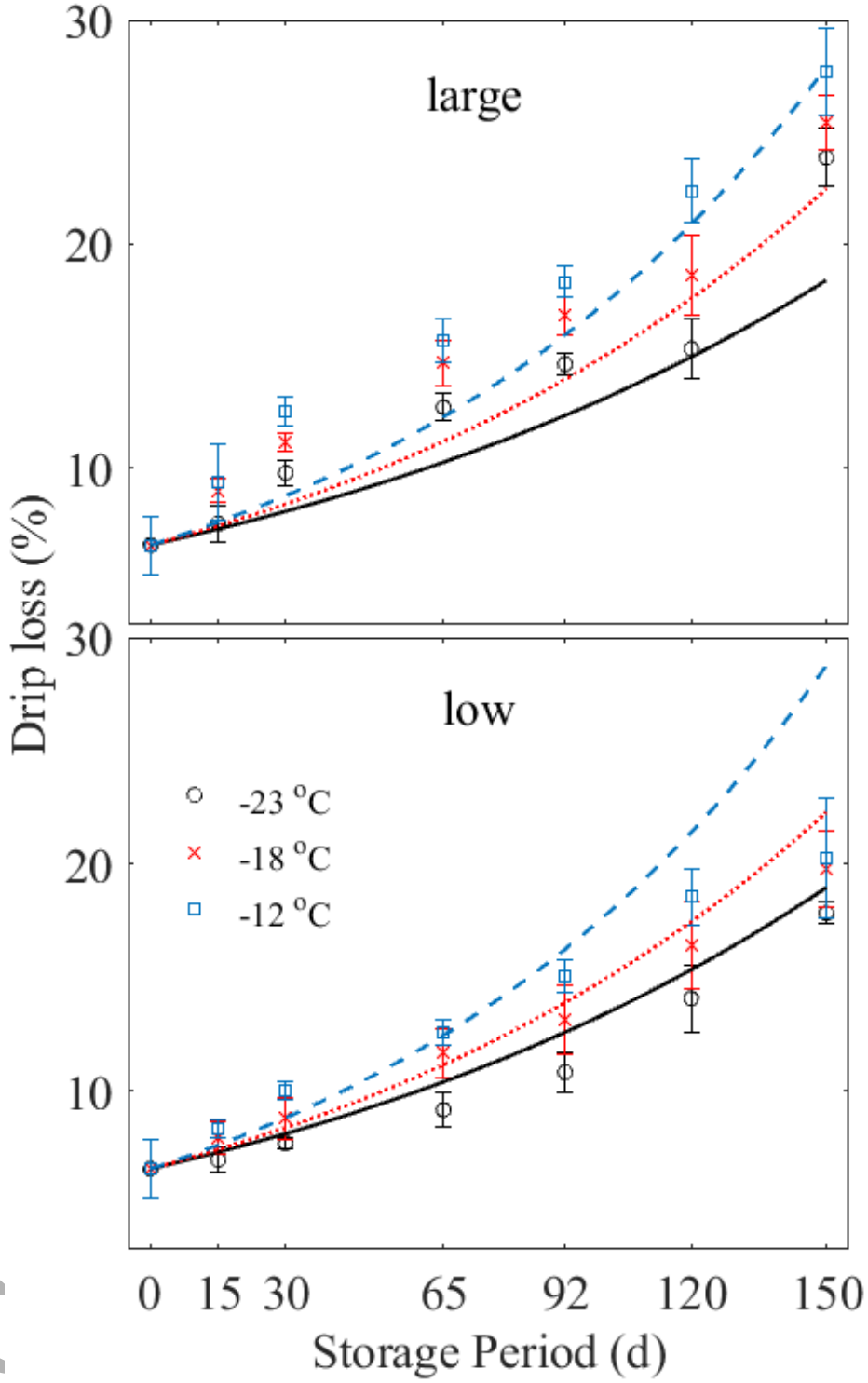

Fig. 5. Effect of mean temperatures with low and large temperature fluctuations on thawing drip loss (%) of the frozen apple tissue during frozen storage under fluctuating temperature regimes. The lines are the fitted output of drip loss. The points are the means of the measured values from three replicates, with error bars representing the standard error at each time-point.

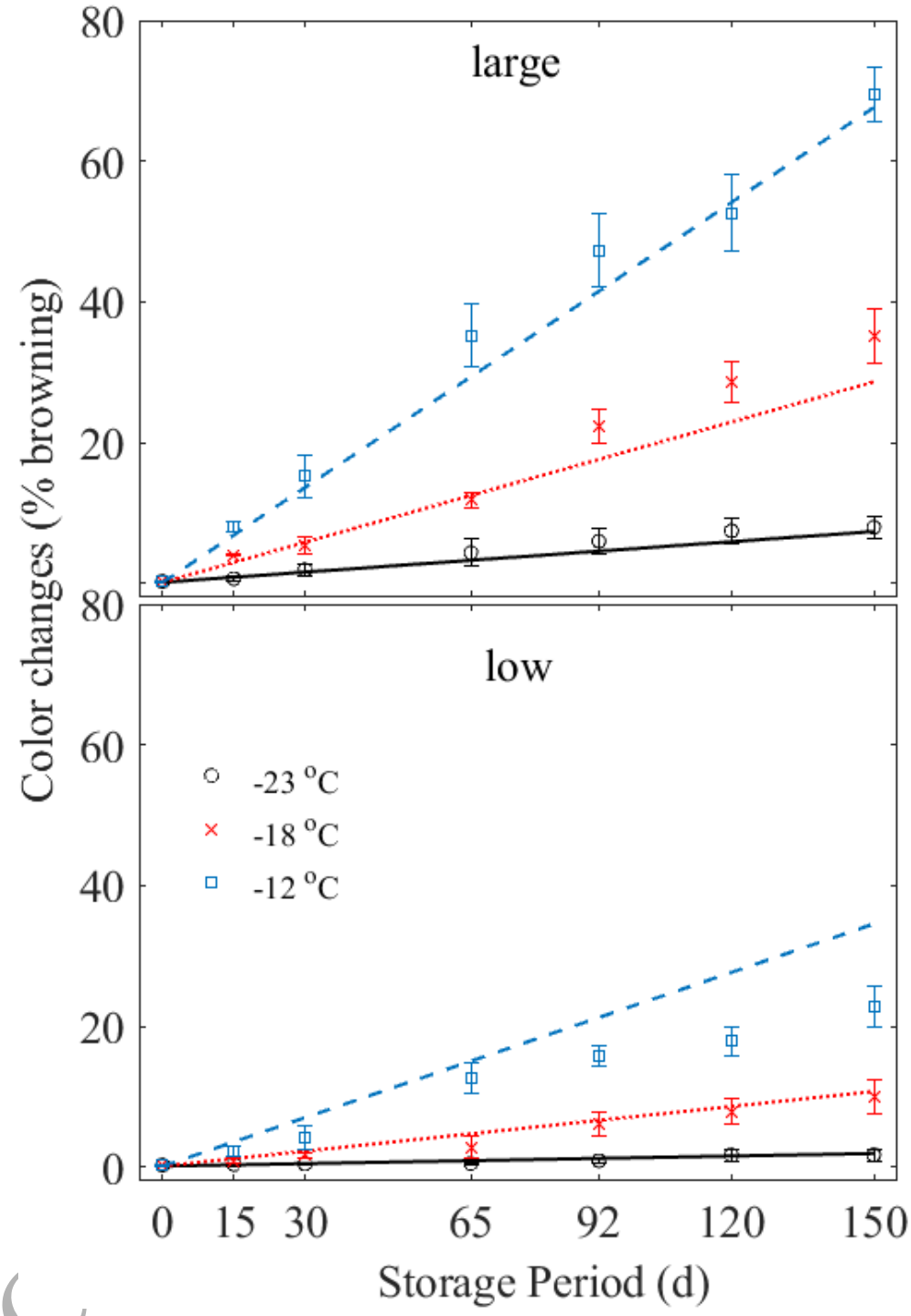

Fig. 6. Effect of mean temperatures with low and large amplitudes of temperature fluctuations on color changes (expressed in % browning) of the frozen apple tissue during frozen storage under fluctuating temperature regimes. The lines are the fitted output for color changes. The points are the means of the measured values from three replicates, with error bars representing the standard error at each time-point.

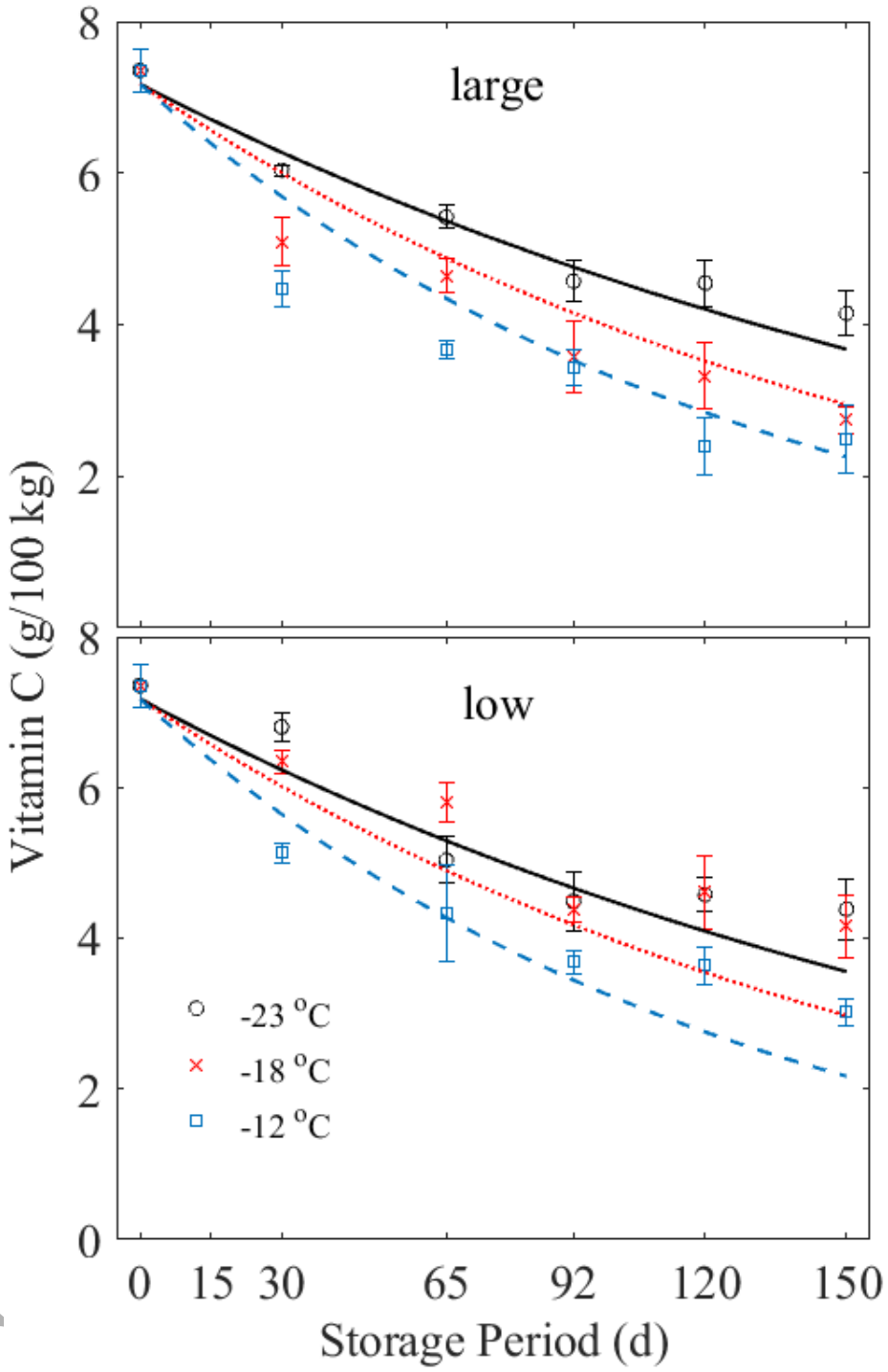

Fig. 7. Effect of mean temperature with low and large amplitudes of temperature fluctuation on vitamin C (ascorbic acid) concentration (g per 100 kg) of the frozen apple tissue during frozen storage under fluctuating temperature regimes. The lines are the fitted data of vitamin C (ascorbic acid) concentration. The points are the means of the measured values from three replicates, with error bars representing the standard error at each time-point.

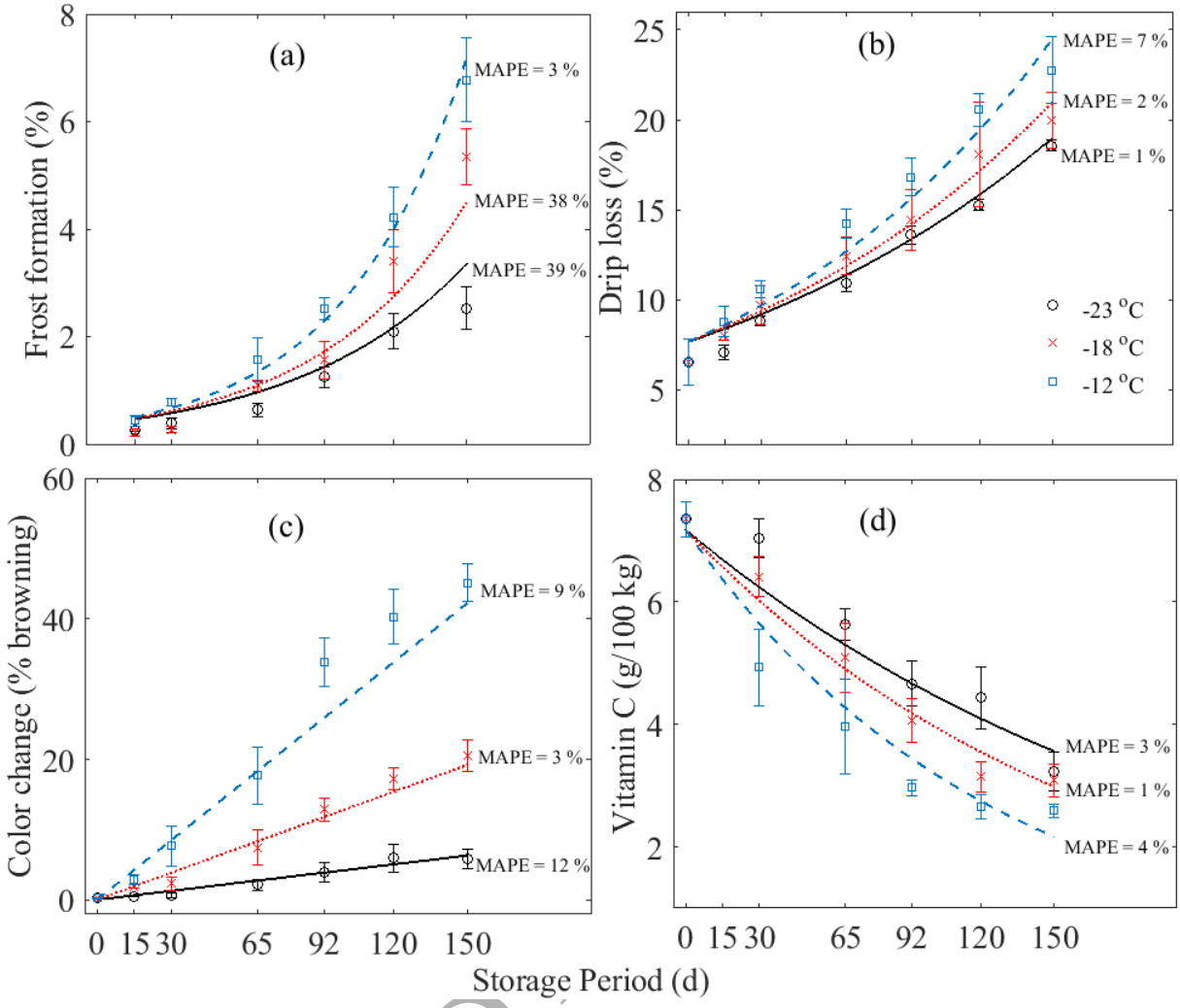

Fig. 8. Predictions of the quality changes: (**a**) frost formation, (**b**) drip loss, (**c**) color changes and (**d**) loss in vitamin C (ascorbic acid) content in apple tissue stored at different temperatures with medium amplitude of temperature fluctuation. The lines outline the predicted values, while points are the means of the measured values for each quality attribute, with error bars representing the standard error at each time-point. MAPE (%) is the mean absolute percentage error between the predicted and measured values.

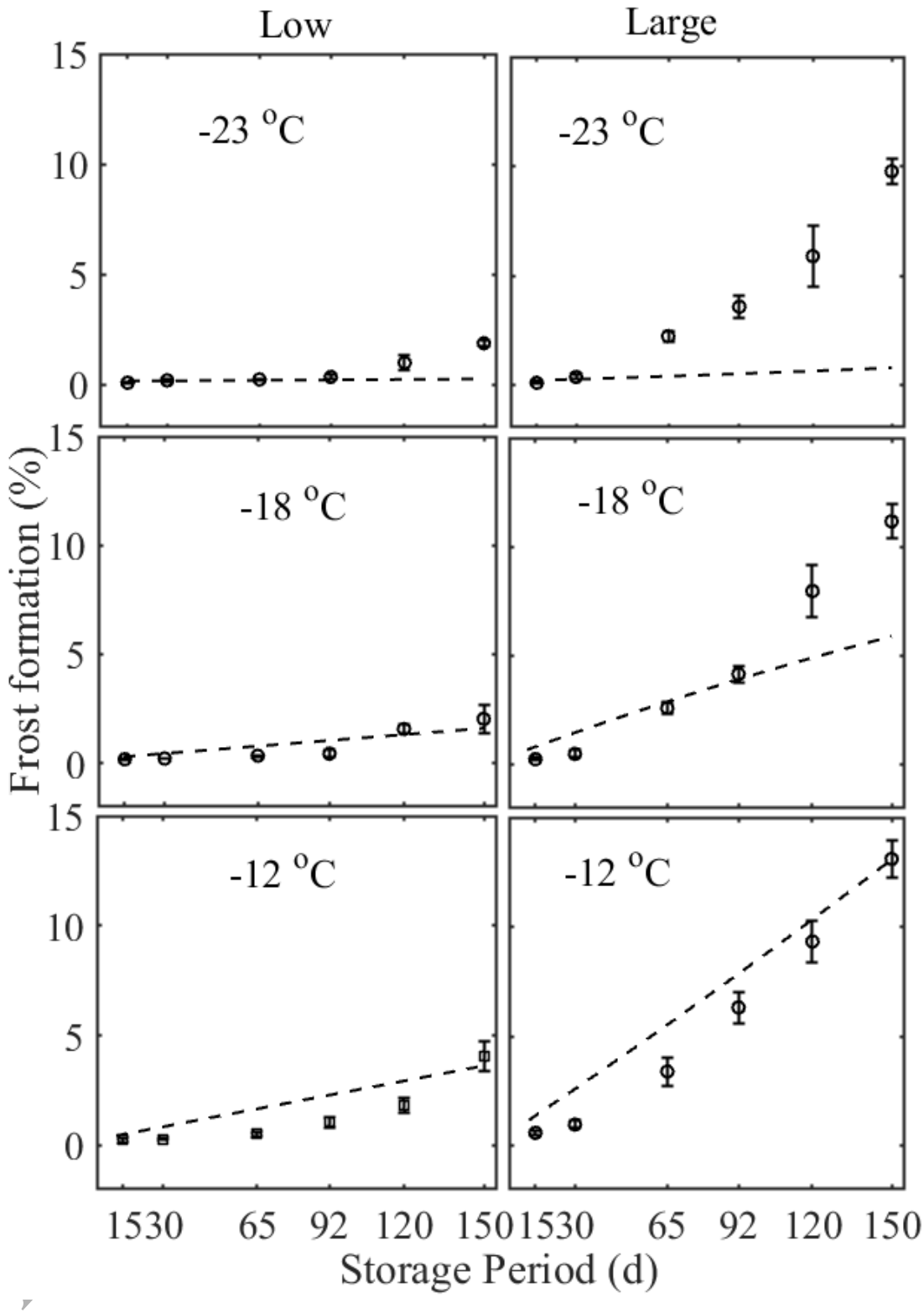

Fig. 9. Model fitting of frost formation (%) using the physical model Eq. (7) in apple tissue stored with low and large amplitudes of temperature fluctuations. Dotted lines are the fitted data for frost formation. The points are the means of the measured values from four replicates, with error bars representing the standard error at each time-point.

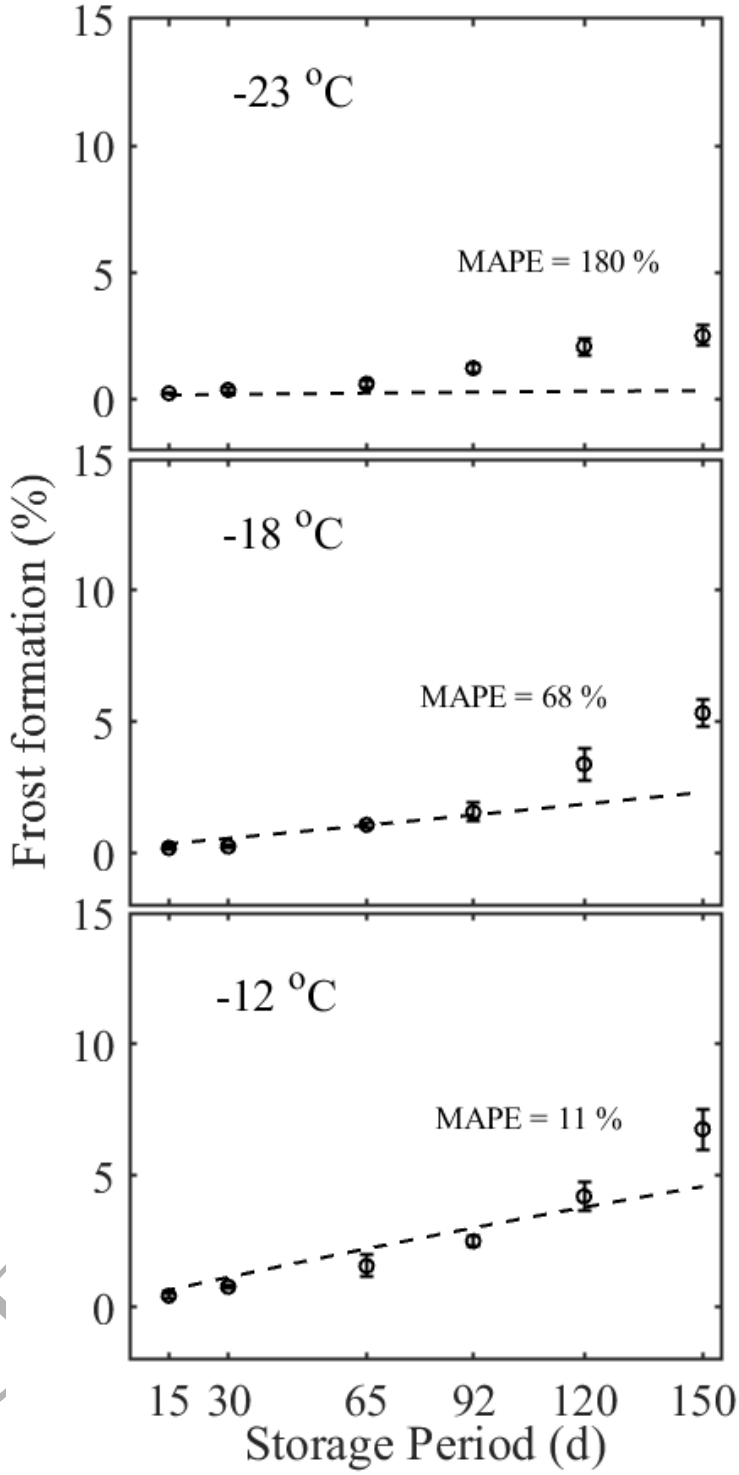

Fig. 10. Predictions of frost formation using the physical model Eq. (7) in apple tissue stored at different temperatures with medium amplitude of temperature fluctuation. Dotted lines are the predicted values, while points are the means of the measured values from four replicates with error bars representing the standard error at each time-point. MAPE (%) is the mean absolute percentage error between the predicted and measured values.

Table 1: Kinetic model parameter estimates, together with the standard deviations (S.D.) for quality changes (frost formation, drip loss, color changes and loss in vitamin C (ascorbic acid) content) in frozen apple tissue during five months of storage at -12, -18, and -23 ^{o}C all at low and large.

Quality indicators (Q)	**[a]Parameter estimate values**		
	Q_0	**$k_{ref,i}$ (% d^{-1} or d^{-1})**	**$E_{a,i}$ (kJ mol^{-1})**
Frost formation (%)	0.27 ± 0.04	0.017 ± 0.001	14.0 ± 1.4
Drip loss (%)	7.67 ± 0.25	0.0068 ± 0.0003	11.7 ± 2.1
Color changes (%)	0.44 ± 0.03	0.0088 ± 0.0019	22.5 ± 3.0
Vitamin C (mg per kg)	71.8 ± 3.1	0.0059 ± 0.0002	25.3 ± 3.3

[a]$k_{ref,i}$ are the rate constants for each quality indicator (all rate constants are giver for a reference temperature of -18 ^{o}C), and $E_{a,i}$ are the activation energies for each quality evolution.