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1. Aim

The aim of deliverables 3.2.4.1-3.2.4.4 is to review the tools/methodologies cited in the literature for predicting the evolution of the temperature dependent quality attributes and microbial growth in refrigerated/frozen food for a constant storage temperature or under dynamic conditions. These tools/methodologies will, later on in the FRISBEE project, also evaluate the effect of random fluctuations of refrigeration temperatures on quality and microbial load. The tools will be used for evaluation and optimisation and to develop monitoring and control strategies. The present deliverable 3.2.4.4 comprises a compilation and evaluation of existing microbiological and quality kinetic models and data related to spinach and ice cream under frozen conditions. It is based on an extended literature search with respect to quality and microbiological stability issues for the food products under consideration.

2. Introduction

Most foods have a short durability, and are exposed to conditions that destroy their superior quality in a short period of time, before consumption. Seasonality and perishability of foods 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.

2.1. Vegetables

Vegetables are an important component of the European diet. They provide not only textural and colour variety to the main meal of the day, but often complementary nutrients such as dietary fibre and certain vitamins and minerals. 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. (Giannakourou & Taoukis, 2003). Freezing is a suitable method of processing seasonally harvested vegetables. This is reflected in the steady rise in frozen vegetable consumption over recent years (Martins and Silva, 2003). As Kmiecik et al. (1999), and Lisiewska et al (1996 and 1997) report, frozen products retain greater amounts of almost all chemical constituents compared with canned food, even after preparation for consumption by cooking in water (Lisiewska et al., 2009). One of the vegetables considered to have a high nutritional value is spinach (*Spinacia oleracea L.*). Spinach is often consumed fresh or stored frozen after cooking in boiling water. Frozen spinach is preferred by most consumers due to its prolonged shelf-life which enables it to be available throughout the year (Bunea et al., 2008).

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). The most important quality changes occurring during storage of frozen vegetables are changes in sensory characteristics, such as colour and texture that influence consumer acceptability, transformation of pigments and reduced nutritive value, mainly in vitamin C.

2.2. Ice cream

Ice cream is a frozen emulsion consisting of three phases. Its continuous phase consists of unfrozen syrup (mostly sugars and minerals) while its disperse phase consists of air cells, globules of milk fat, ice crystals and insoluble substances (proteins and hydrocolloids). The major components of ice cream are milk fat, milk proteins, sugar and corn syrup solids. Minor ingredients are vegetable gums and mono- and diacylglycerols (Marshall, 2003a).

As a frozen dessert, the physical characteristic of ice cream is weight per unit volume of the product, which is affected by the overrun (the percentage increase in the volume of the initial ice cream mix caused by the beating-in of air) developed in the product. Hardness of the product at the temperature of dripping is of high significance. Hardness is affected by several factors such as melting point, total solids, overrun and stabilizer type and concentration (Marshall, 2003b).

The ingredients used in the manufacture of ice cream have certain effects on its chemical and physical properties and functionality, as they contribute to the microstructure of the product. Much work has focused on the contributions of ingredients or their components towards studying and controlling colloidal properties and microstructure (ice recrystallization, glass transition and development of stabilizers). The latest developments in the area of ice structuring protein from natural sources, have focused on understanding and controlling of fat destabilization and emulsifier functionality, and enhancing knowledge of the functionality of protein at the air bubble interface and stability (Goff, 2008).

Most consumers prefer ice cream that feels smooth and moderately firm when eaten. The degree of smoothness depends on the sizes of the substances suspended in the disperse phase of the product. Particles that are 0.1-2 μm in diameter impact creaminess to ice cream while particles larger than 3 μm cause gritty texture, (Marshall, 2003b). (After hardening the average ice crystals diameter is much higher that 3 μ) The limit of crystal diameter for acceptable mouthfeeling is approximately 40μ.

A number of works on the effects of storage conditions for ice cream have been conducted in the last 15 years, including Flores and Goff (1999) and Donhowe and Hartel (1996a,b). Storage conditions govern mainly the final quality of ice cream. The most frequently occurring textural defect in ice cream is the development of a coarse, icy texture that probably accounts for lost sales through customer dissatisfaction with quality (Goff, 2008). Shelf-life of ice cream might be one year, or two weeks or less depending on its storage conditions such as temperature and temperature fluctuation.

Texture is affected by the melting point of the unfrozen dissolved phase of ice cream. Ice cream should melt to a smooth and uniform consistency within about 20 minutes at room temperature. Furthermore, adjusting an ice cream formula to produce slow melt can cause slow release of delicate flavors. A high concentration of dissolved substances results in a low freezing point, a relatively small amount of ice crystals formed, and soft ice cream. In contrast, low concentrations of dissolved substances result in hard ice cream. Stabilizers and emulsifiers also affect texture. Stabilizers bind water, reducing the amount available to form ice crystals, and increase the viscosity of the continuous phase. Emulsifiers contribute to formation of small air cells as well as moderate clumping of the fat. When used in certain amounts, emulsifiers provide stability to the emulsion and a more slow rate of melting (Marshall, 2003b).

3. Frozen vegetable quality and shelf-life

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 (Kramer, 1974; Labuza, 1982; Martens, 1986; Hung & Thomson, 1989; Lisiewska & Kmiecik, 1996, 1997; Oruña-Concha et al., 1998; Kmiecik & Lisiewska, 1999; Pilar Cano, 1999). Recent studies report vitamin C contents of several frozen green vegetables (Haag et al., 1995; Lisiewska and Kmiecik, 1997; Favell, 1998; Howard et al., 1999; Kmiecik & Lisiewska, 1999; Giannakourou & Taoukis, 2003; Martins & Silva, 2004a,b; Berger et al., 2007; Tosun & Yücecan, 2008) and the effect of pretreatments and storage temperatures on the preservation of ascorbic acid.

Overall quality and shelf life of frozen vegetables is highly correlated to sensory characteristics, such as colour and texture that influence consumer acceptability, transformation of pigments and reduced nutritive value, mainly in vitamin C. The rates of the above concurrently occurring actions depend on storage temperature. The current effort is to develop and apply a systematic kinetic and modelling approach to the main quality indices of each product (Martins & Silva, 2004a,b; Giannakourou & Taoukis, 2003; Hertog et al., 2007; Gonçalves et al., 2009). There is a significant number of published data with regards kinetic study of frozen vegetables such as green beans (Giannakourou & Taoukis, 2003; Martins & Silva, 2004a,b; Martin et al, 2004, 2005), grean peas, spinach and okra (Giannakourou & Taoukis, 2003), tomato (Dermesonlouoglou et al., 2007) and cucumber (Dermesonlouglou et al., 2008). Most research has been performed under stationary (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 the frozen products' commercial shelf-life) temperature conditions to obtain model parameters of the quality degradation kinetics. 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 (Giannakourou & Taoukis, 2002, 2003b). 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.

Storage temperature and variability determine quality degradation rates and shelf life at the time of consumption or use. Since storage temperatures for frozen vegetables in commercial handling and distribution vary, it is important to be able to reliably estimate the effect on shelf life under variable temperature conditions that is likely to occur in the actual frozen food chain.

Published information on systematic modelling of the temperature dependence and validation in variable conditions relevant to real cold chain conditions would be important for shelf life optimization and improvement of the cold chain management (Giannakourou & Taoukis, 2001, 2003; Martins et al., 2005; Laguerre & Flick, 2007). For many food products, - fruit and vegetables in particular- quality models are neither available, nor validated for dynamic refrigeration conditions nor implemented in user-friendly software environments. In addition, the dependency of quality and microbial kinetics on temperature implies that for certain foods the temperature distribution inside the food (rather than just on the surface) could be taken into account. Today, no such integrated evaluation tool is available or validated for refrigerated foods. For frozen foods, however, temperature profiles within the volume of the food are more uniform due to the much higher thermal conductivity in the frozen state.

4. Frozen spinach quality and shelf-life modelling under static and dynamic conditions

4.1. General quality characteristics of frozen spinach

One of the vegetables considered to have a high nutritional value is spinach (*Spinacia oleracea L.*). It contains considerable amounts of vitamins, especially C, E, β-carotens, folic acid, mineral components and dietary fibres (Lisiewska et al., 2009). Among the vegetables it is also characterized by its high antioxidative capacity (Jaworska, 2005). Frozen spinach is preferred by most consumers due to its prolonged shelf-life which enables it to be available throughout the year. Its value as raw material for freezing has been confirmed by numerous data in the literature (Lisiewska & Kmiecik, 1997; Kmiecik and Lisiewska, 1999; Jaworska & Kmiecik, 2000).

The stability of carotenoids in spinach subjected to different storage conditions has been addressed in limited reports (Kopaslane & Warthesen, 1995; Bergquist et al., 2006) (Table 1). Bunea et al. (2008) found that among individual carotenoids, lutein is the most stable, followed by β-carotene, while violaxanthin being polar and soluble becomes suspectible to degradation. The total amount of spinach phenolic compounds decrease during processing while individual phenolic acids behave differently. The three major phenolic acids found in spinach, namely para-coumaric, ortho-coumaric and ferulic acid, gradually increase during refrigeration, blanching and boiling processes, which demonstrates that not only the stability of these compounds but also the extent of their release from the vegetable matrix due to processing is essential for evaluation. The storage and minimal preparation techniques such as blanching as well as boiling can significantly affect the level and stability of carotenoids and phenolic acids in spinach (Gil et al., 1999; Turkmen et al., 2005; Bunea et al., 2008) (Table 1). Murcia et al. (2009) reported no alteration in antioxidant activity of frozen spinach stored at domestic freezer conditions (-20°C) even after storage for 8 months. Lisiewska et al. (2009) studied the retention of ash and 11 minerals (ash, P, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, Cr, Ni) in ready to eat frozen spinach after 12 months of storage at -20C and concluded that there was no consistent pattern with regards the remaining concentration of the elements (Table 1). Limited experimental data have appeared concerning the microbiology of frozen spinach (Smart & Brunstetter, 1937). To preserve frozen vegetable nutritional value and to assure the food safety, high quality raw material is necessary (Ninfali and Bacchiocca, 2004). Hall and Alcock (1987) noted that vegetables continue to deteriorate during frozen storage when high levels of microbial population are reached before freezing. However, spoilage during storage occurred at levels where the initial quality had not been reduced and thus the deterioration was due to pre-formed microbial enzymes. Such spoilage could be expected when the population before freezing reaches a level of logCFU/g equal to 8.0. Good hygiene handling and low storage temperature are significant factors for assuring no growth rate of spoilage microorganisms in the frozen packs of these products (Table 1).

Table 1. Published data on frozen spinach.

Food sample	**Processed**	**Storage conditions (Temperature, Time)**	**Quality indicator**	***Reference***
Spinach (Spinacia oleracea) Var. Leopold leopard, Belgium	Blanched for 2 min in boiling water at 100°C, & Blanched, frozen, stored, and then boiled 10 min in water	-18°C, 1 month	Neoxanthin, Vioxanthin, Lutein, β-Carotene, & Total carotenoids	*Bunea et al., 2008*
Spinach (Spinacia oleracea) Var. Leopold leopard, Belgium	Blanched for 2 min in boiling water at 100°C, & Blanched, frozen, stored, and then boiled 10 min in water, Fresh	-18°C, 1 month	Ortho-Coumaric acid Ferulic acid Para-Coumaric acid, & Total phenolic acids	*Bunea et al., 2008*
Spinach Germany	Garden fresh, market fresh, supermarket fresh	-18°C, 3, 6, 12 months	Ascorbic acid	*Favell, 1998*
Spinach (Spinacia oleracea)	Fresh, & Blanched at 90°C for 2 min	-5, -10, -15, -20°C (kinetic study of the degradation)	L-Ascorbic acid	*Giannakourou & Taoukis, 2003*
Spinach thick-leafed var. Heavy Pack	Fresh, stored, frozen, before & after cooking	-40°C (no further frozen storage)	Ascorbic acid, Thiamin, Riboflavin, Carotene	*Gleim et al., 1944*
Spinach (Spinacia oleracea) & New Zealand spinach (Tetragonia expasna Murr.)	Blanched at 97-98°C & cooked in brine, Cooked in 2% brine before freezing, &heated in microwave	-20°C, 12 months	Ash P, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, Cr, Ni	*Lisiewska et al., 2009*
Spinach (Spinacia oleracea) var. Balady)	Blanched in hot water or 0.1% MgCO3 for 1 min at 90°C	-18°C, 1, 2, 3 months	Chemical composition (Moisture, total solids, oh, ash) Chlorophyll α, Chlorophyll β, & Total chlorophyll content Ascorbic acid Sensory characteristics (color, flavor, overall acceptability)	*Labib et al., 1997*
Spinach (Spinacia oleracea, L.)		-20°C, 8 months	Antioxidant capacity (Hydroxyl Radical Scavenging Ability) Total antioxidant activity (TEAC, Trolox Equivalent Antioxidant Capacity)	*Murcia et al., 2009*

Colour change of frozen spinach is one of the quality deterioration indices determining spinach shelf-life. Browning caused by polyphenoloxidase (PPO) or change of green or yellow colour to red caused by hydroperoxides produced by the action of lipoxygenase on polystaurated fatty acids are the main causes for colour alteration. Textural changes are caused primarily by the polygalacturonase (PG) although cellulases and hemicellulases may also be involved. The enzymes responsible for loss of desirable flavor and aroma or formation of undesirable flavor are more difficult to pinpoint because of the complexity of flavor. The study of processing and storage effects on spinach flavour, aroma, colour and texture has been conducted by sensory evaluation tests (Labib et al., 1997; Giannakourou & Taoukis, 2003).

Objective instrumental quantification of the colour change based on measurement of CIELab values with the use of chromatometer has been successfully applied for monitoring frozen vegetable quality loss (Giannakourou & Taoukis, 2002; Martins & Silva, 2002, 2003) (green peas, and green beans, respectively). Alternatively, the colour change for green vegetables has been expressed measuring the chlorophyll a and b, and pheophytin a and b contents (Martins & Silva, 1998, 2002). As far as the texture of the frozen vegetable tissue is concerned, it has been expressed -instrumentally- with the use of texture analyzer measurements and water holding capacity measurements (the change of weight due to water absorbed or adsorbed) (Giannakourou & Taoukis, 2002; Martins & Silva, 2002, 2003). However the published data could not be correlated significantly with time and temperature to allow shelf life estimation of frozen spinach .

4.2. Kinetic modeling of Vitamin C in frozen spinach

Processing and distribution and storage of frozen spinach are detrimental factors for ascorbic acid retention, since it is oxidized to dehydroascorbic acid, which is irreversibly hydrolysed to 2,3 diketogulonic acid,. This oxidation is enhanced by temperature abuses during frozen storage. The retention of ascorbic acid in frozen products is thus strongly dependent on their storage temperature profile (Favell, 1998; Martins & Silva, 2003). According to Favell (1998), blanching and freezing processes led to a loss of initial vitamin C retention for spinach equal to 78 and 58%, respectively. A further 30% loss occurred after 12 months of deep frozen storage. The samples stored at ambient temperature lost ascorbic acid very rapidly with only 10% remaining after 3 days. The chill sample lost ascorbic acid less rapidly, but even so only 20% remained after 7 days, falling to zero before day 14.

The level of Vitamin C has been used, in the case of frozen spinach, as a reliable and representative index for estimating quality deterioration during frozen storage and estimation of shelf life (Figure 1, Table 2 in Giannakourou & Taoukis, 2003). Vitamin C is determined by a high performance liquid chromatography method (HPLC) (Giannakourou and Taoukis, 2003), or a 2, 6 dichloroindophenol titrimetric method ([AOAC Official methods of analysis, 1984], 43.064). All analyses are carried out in homogenized vegetable tissue. Five grammes of homogenate are mechanically stirred in 15 ml of a 4.5%(w/v) solution of metaphosphoric acid for 15 min. The mixture is vacuum-filtered and diluted with HPLC grade water; the total final volume is measured and an aliquot is filtered through a 0.45-μm filter prior to injection into the chromatographic column. The mobile phase is HPLC grade water with metaphosphoric acid to pH 2.2; detection at 245 nm. Accelerated storage tests have been a widely used method to assess the shelf life of frozen vegetables. Vitamin C content of spinach is a quantifiable nutritional index that is indirectly linked to quality. A correlation of this index to sensorially important indices that determine consumer quality (colour, texture etc) is important for shelf-life determination.

The average retention of ascorbic acid (L-ascorbic acid) is expressed relatively to an initial, average value of day 0 of the experiment, 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.1):

$$C = C_0 e^{-kt} \text{ or } \ln\frac{C}{C_0} = -kt \qquad (1)$$

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. This linearized plot is valid only under constant conditions of temperature, i.e., where k does not depend on time.

After the freezing/blanching process, during the subsequent isothermal frozen storage, leafy spinach exhibited a first order loss of Vitamin C at all temperatures studied (Fig. 1a). Temperature dependence of Vitamin C deterioration was expressed with the Arrhenius equation (Eq. 2) (Fig. 1b) 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} value (Q10 is the increase in the rate of a deterioration factor when the temperature is increased by 10°C) for the range –15 to –5°C, are shown in Table 2.

$$k = k_{ref} \exp\left[\frac{-E_A}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \tag{2}$$

In Eq. 2, 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 ($1/T_{ref}$-1/T) (Arrhenius plot), the E_A of L-ascorbic oxidation can be estimated from the slope of the fitted line. To demonstrate the integrated effect of the temperature variability on product quality, the term of the effective temperature T_{eff} has been introduced by Giannakourou and Taoukis (2003). T_{eff} is defined as the constant temperature that results in the same quality value as the variable temperature distribution over the same time period, based on the Arrhenius model and integrates in a single value the effect of the variable temperature profile.

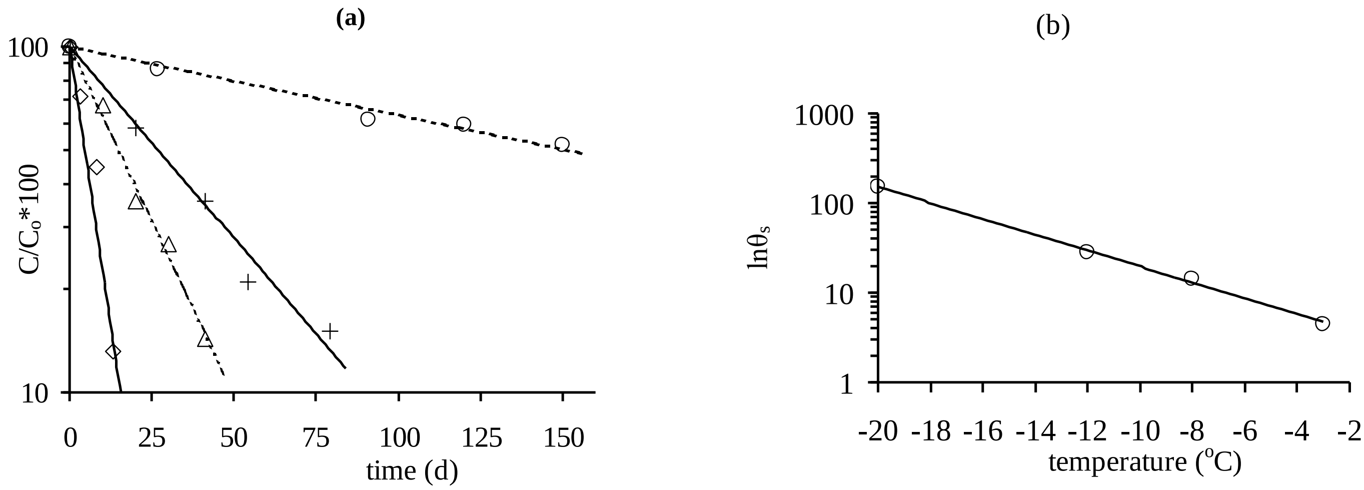

Figure 1. (a) Results for Vitamin C loss vs time at 4 storage temperatures in a semilogarithmic scale. Experimental points correspond to: ◊ at –3°C, Δ at –8°C, + at –12°C and o at –20°C and lines represent the first order fit (R2 > 0.972 at all temperatures) (b) Shelf life (θ) plot of the 50% Vitamin C loss for frozen spinach in a semilogarithmic scale. Based on data from (Giannakourou and Taoukis (2003)).

Table 2. Arrhenius parameters and statistics, Q_{10} values and shelf life at four temperatures in the frozen storage range for frozen spinach (Giannakourou & Taoukis, 2003).

Arrhenius parameters and statistics, Q_{10} values for frozen spinach	
E_A (kJ/mol)	111.9±23.2
k_{ref} (1/d)	0.00454
R^2	0.992
Q_{10} (in the range –15 to –5°C)	7.0
Shelf life (days) is based on 50% Vitamin C loss	
-5°C	8
-10°C	20
-15°C	55
-20°C	153

The established kinetic models were validated at dynamic storage conditions in programmable freezer-incubators. The nutritional change under variable temperature conditions T(t) for time t_{tot} can be calculated by Eq. 3.

$$\ln\left(\frac{C_{t_{tot}}}{C_o}\right)=\int_0^{t_{tot}} k(T(t))dt=k_{eff}\,t_{tot} \quad (3)$$

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 or is discretized to small time increments t_i of constant temperature T_i, where $\Sigma\, t_i=t_{tot}$, then Eq. 3 can equivalently be expressed as:

$$k_{ref}\sum_i\left(\exp\left[-\frac{E_A}{R}\left(\frac{1}{T_i}-\frac{1}{T_{ref}}\right)\right]t_i\right)=k_{eff}\,t_{tot} \quad (4)$$

from which k_{eff} can be estimated. For $k=k_{eff}$, the value of the effective temperature T_{eff} can be calculated from the Arrhenius equation.
In Fig. 2, measurements of Vitamin C 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 spinach. Repeated temperature cycles included three step changes as shown in the same Figure. The exact time-temperature sequences used are reported in Table 3.

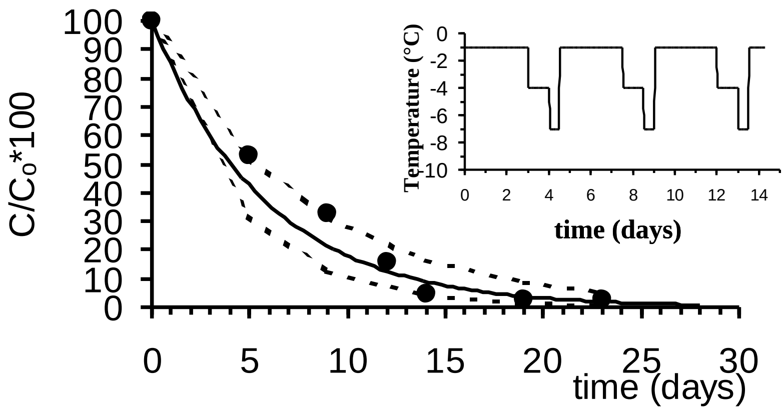

Figure 2. Comparison of experimental (closed circles) and predicted results of Vitamin C loss of spinach for exposure at the shown variable temperature profile. The solid line represents the exponential fit of the quality measurements and dotted lines depict the upper and lower 95% confidence range of quality predicted for T_{eff}. From Giannakourou and Taoukis (2003).

Predicted rate of loss, k_{eff}, are in good agreement with the experimentally estimated ones, k_{exp}, as demonstrated in Table 3, where the 95% confidence intervals of k_{eff} , the goodness of fit and the ±95% confidence range of k_{exp} (using the standard *t*-value) are also calculated. Since the estimated value of k_{exp} falls within the 95% confidence intervals of k_{eff}, the two rates are considered statistically equivalent in non-isothermal experiments conducted for frozen spinach.

Table 3. Time-temperature conditions of the repeating cycles and comparison of experimental (k_{exp}) vs predicted (k_{eff}) rate of Vitamin C loss for the non-isothermal experiments conducted for frozen spinach.

Time-temperature conditions of repeating cycles	**1st stage: 72 h at -1°C 2nd stage: 24 h at -4°C 3rd stage: 12 h at -7°C**
T_{eff} (°C)	-1.7°C
k_{eff} (1/d)	0.1726±0.0427[1]
k_{exp} (1/d)	0.1640±0.0545[2]
R^2 for k_{exp}	0.917

(Giannakourou & Taoukis, 2003)

Giannkourou and Taoukis (2003), besides frozen spinach studied three other frozen vegetables, namely green beans, green peas and okra. With the validated kinetic equations for each vegetable, their remaining shelf life can be predicted at any point of their distribution, from manufacture to consumption. For this purpose, a realistic distribution scenario in the current chill chain has been assumed for spinach, green beans, green peas and okra. This scenario includes an initial stage of 10 days storage in the factory warehouses, intermediate transport, followed by a 10 days stocking in the wholesale stage (or alternatively in a distribution center). Subsequently, vegetables have been transported and exposed at retail freezers for 20 days, before being purchased by the final consumers that keep them stored for 20 days before final cooking and consumption (Giannakourou and Taoukis, 2002). 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.

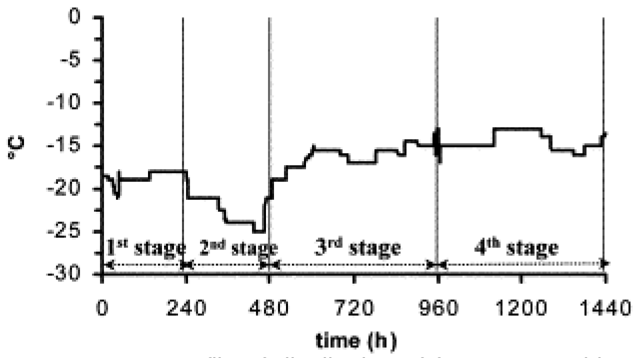

Fig. 3. Indicative temperature profile of distribution of frozen vegetables in the real chill chain (total distribution time 60 days) (Giannakourou and Taoukis, 2003).

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 Eq. 3 and Eq. 4. To calculate f_{con}, the fraction of shelf life consumed at each stage, the time/temperature/tolerance (TTT) approach (Labuza & Fu, 1997) can equivalently be used. 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:

$$f_{con} = \sum \frac{t_i}{\theta_i} \qquad (7)$$

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 whole marketing route (60 days after production), at the time of consumption spinach has lost more than 50% Vitamin C, under the specific temperature conditions. It is worth noting that in the case of spinach, the short shelf life (based on Vitamin C) at all temperatures (Table 2) demonstrates the sensitivity of this vegetable to Vitamin C loss, when compared to other frozen vegetables. Thus although Vitamin C is a good quantifiable index for the effect of temperature storage, commercial shelf life should also be based also on other indices important and perceptible by the consumer such as colour or chlorophyll degradation.

The assumption that the deterioration process can be characterized by a single rate constant is limited. A first order reaction rate may be used for deterioration process characterization. The use of the Arrhenius equation requires that under unchanged conditions, e.g. constant pH, moisture content, the rate constant, however defined, is only a function of temperature and hence totally unaffected by the food's thermal history. Additionally, fitting of isothermal decay data can be done using any of the many commercial software packages available. The Weibullian model is one example, used by Corradini and Peleg (2006) for degradation of vitamin C in frozen spinach.

$$\Upsilon(t) = \frac{C(t)}{C_O} = \exp(-bt^n) \tag{8}$$

The one below is another empirical model.

$$\Upsilon(t) = \frac{C(t)}{C_O} = 1 - \frac{t}{k_1 + k_2 t} \tag{9}$$

In both models C(t) and C_0 are, respectively, the momentary and initial concentrations of the monitored compound, such as a vitamin, pigment etc. The constants, b and n, or k_1 and k_2 are temperature dependent parameters. The first order kinetic decay is just a special case of Eq. (8) where n=1. The zero order kinetics is just a special case of Eq. (8) where n=0. Thus, all that follows will also apply to zero and first order kinetics if and when they are encountered but not vice versa.

In conclusion, published work is available with regards modeling frozen spinach shelf-life. Vitamin C has been considered as the main deteriorative factor for this kind of product. Nevertheless, the spinach vitamin C retention has to be correlated with other quality indices such as colour, texture etc important to the consumers to evaluate the remaining shelf-life. Thus in the context of WP3, optimally designed storage experiments will be conducted at isothermal and dynamic conditions to validate the shelf life models under "real" distribution and storage conditions scenario based on data introduced in the database on cold chain in Europe developed in WP2. These experiments besides vitamin C will include in parallel and correlate other important quality indices (colour, chlorophyll degradation, sensory scoring). Variability in factors that may affect vitamin C loss and shelf life, such as pH and moisture content of the product will be introduced in the equations. The models that will be developed will be applicable for frozen spinash products of varying characteristics and for the dymanic conditions of the cold chain.

5. Ice cream texture and recrystallization

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Many published studies are dealing with the improvement of the microstructure of frozen desserts such as ice cream. These studies focus on the use of different stabilizers and emulsifiers, of fat replacement by modified substances, and on the modification of functionality of the final products (by adding probiotics and/or prebiotics), and their effect on microstructure, rheology, functional properties, and sensory characteristics of ice cream (Bolliger et al., 2000; Aime et al., 2001; Kaya & Tekin, 2001; Innocente et al., 2002; Ganger et al., 2003; Patmore et al., 2003; Wildmoser et al., 2004; Zhang & Goff, 2004; Vega & Goff, 2005; Soukoulis et al., 2008; Soukoulis et al., 2009; Soukoulis et al., 2010a; Soukoulis et al., 2010b).
The main texture parameters describing the ice cream are the firmness, the hardness and the viscosity. The firmness of ice cream is related to its structure. The air cells of ice cream structure are essentially spherical although there is some distortion due to fat and ice crysral formation (Prentice, 1992). The material surrounding these air cells is a non-Newtonian fluid containing, primarily, clumps of fat (up to 80%) and small ice crystals. In fat reduced ice cream products, it is clear that the rheology of the composite fluid surrounding the air cells wiil be altered due to the reduction in the fat clumps which predominate the composite fluid of

conventional ice cream structure. The instrumental analysis of ice cream firmness and apparent viscosity should provide some insight into the foam structure of fat reduced ice crearn products.
A physical property of ice cream that has a major influence on sensory quality in general, and texture assessrnent in particular, is apparent viscosity. Apparent viscosity in the partially melted state is an important factor because it influences how a sample of ice cream reacts within a person's mouth. The resistance of ice cream to the mechanical forces imparted by the tongue, upper palate, and teeth, will dictate the overall perception of ice cream texture.

Hardness (i.e., the resistance of ice cream to deformation by an external force) is a major physical attribute of ice cream and one of the factors that determines the quality of ice cream: ice cream that is too hard is considered less optimal because of the difficulties associated with its scoopability. Ice cream hardness can be controlled in one of two ways. The first is to control the ice cream formulation, and many researchers have studied the effect of food ingredients on hardness. The second way is to control process conditions.
Temperature fluctuations appearing due to global shipments of ice cream affect ice recrystallization which is recognized as one of the major quality defects in ice cream, limiting its shelf life and bringing forth consumers' complaints, There is a clear trend toward studying various factors related to ice recrystallization in the complex milieu of ice cream rather than in model systems, taking into account structural effects such as stabilizers effect, emulsifier action, fat destabilization, air distribution, and phase separations, interactions that were previously not considered. Novel ingredients are also being suggested to help control ice recrystallization, such as the ice structuring proteins that can be extracted from natural sources (Regand & Goff, 2006).
Much work has focused on the mechanisms of ice recrystallization since it is the main factor of quality degradation of ice cream. The measurement of ice crystal size of ice cream is conducted using an optical microscope (Donhowe et al., 1991), set to a specific temperature. The optical microscope housed within a refrigerated glove box is used to take photomicrographs of ice crystals in each ice cream sample. A few mg of each sample placed on a microscope slide are diluted with a few drops of n-butanol and spread into a thin film by pressing a second microscope slide onto the sample. The n-butanol is used as dispersant to provide adequate dispersion of ice crystals while maintaining initial crystal integrity. Negatives are enlarged and analyzed by image analysis according to procedures described by Donhowe (1993). The rate of ice recrystallization is depending on the storage temperature and the potential temperature fluctuations. This phenomenon has been studied for almost half a century and many mechanisms have been proposed based mainly on Ostwald ripening principles. The first efforts to study and model ice recrystallization were conducted using a thin layer of ice cream mounted on a microscope under controlled accelerated conditions, in a temperature range of -5 to -20ºC. In order to achieve possible temperature fluctuations, sinusoidal oscillations from 0.1 to 2ºC for a time period of 10 minutes to 2 hours were used. Results indicated that the rate of ice recrystallization increased either when the storage temperature was increased or when temperature fluctuations were observed. Storage under temperature with fluctuations, led to ice crystals of irregular shape, high mean size and ice recrystallization followed a melt-refreeze mechanism, while for storage at constant temperatures (maximum temperature fluctuation of 0.01ºC) the ice crystals had small mean size, they were rounded and ice recrystallization followed a rounding mechanism (Donhowe & Hartel, 1996a, b). Even though these studies provide useful information on a major issue concerning ice cream production, the consideration of this product as a thin layer, instead of a three-dimensional object, generates questions on the differences that may occur in the bulk storage of this product.

6. Ice cream quality and shelf-life modelling under static and dynamic conditions

There is limited work cited in the literature concerning the effect of storage temperatures on the texture parameters. Inoue et al., 2009 modeled the effect of freezer conditions on the hardness of ice cream. A central composite face-centered design was used for their experiments, which includes the 5 freezer conditions [mix flow (**Mix**; L/h), overrun (**Ovr**; %), drawing temperature (**Tem**; °C), cylinder pressure (**Cyl**; kPa), and dasher speed (**Das**; rpm)] as factors. They also investigated the effect of penetration at 3 different temperatures -5, -10 and -15C (DP-5, DP-10, and DP-15).

The quadratic model shown below was used for the modeling of hardness with the above mentioned factors. The model parameters are predicted in a least squares multiple regression analysis:

$$Y = b_o + \sum_{i=1}^{5} b_i X_i + \sum_{i=1}^{5} b_{ii} X^2{}_i + \sum_{i=1}^{4} \sum_{j=2}^{5} b_{ij} X_i X_j$$

where *Y* is the response, *Xi* and *Xj* are the levels of the factors, *b*0 is a constant, and *bi*, *bii*, and *bij* are the coefficients for the main effects, the second-order effects, and the interactions, respectively.
Figure 4 shows the contour plots of the response surfaces for each model to incorporate the largest effective parameter of Ovr and the second-largest effective parameter of Tem. All other parameters were fixed at their central points: Mix of 75 L/h; Cyl of 300 kPa; Das of 222 rpm. A comparison of 3 contour plots (Figure 4, panels a, b, and c) shows that the DP-5 model had the largest penetration value, followed by DP-10 and DP-15.

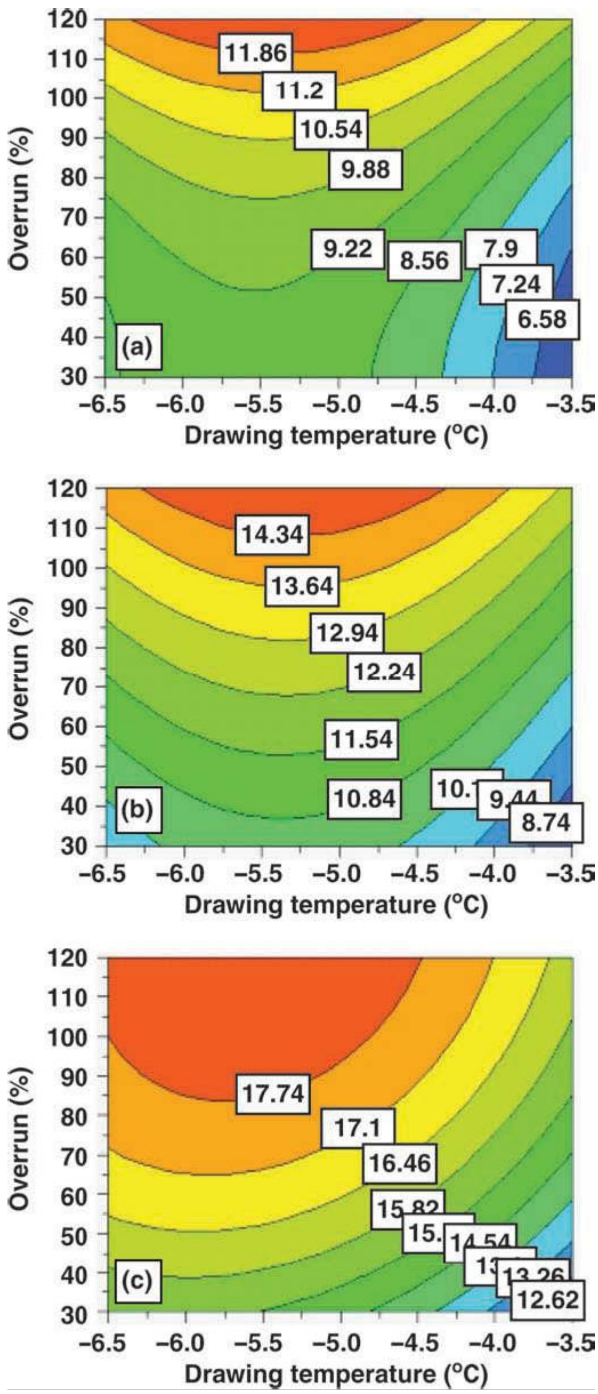

Figure 4. Contour plots of the effect of overrun and drawing temperature on depth of penetration predicted by 3 models: a) DP-15, b) DP-10, and c) DP-5, where DP-15, DP-10, and DP-5 = depth of penetration at –15°C, -10°C, and –5°C. The other process parameters were set at their center values: mix flow rate of 75 L/h, cylinder pressure of 300 kPa, and dasher speed of 222 rpm.

Ice cream viscosity may be measured using parallel disks whose geometry, deformation and shear stress are a linear function of the plate radius r. For oscillatory measurements, maximal deformation γ_R and stress values τ_R occur at the outer radius R assuming Newtonian fluid behavior (Macosko, 1994):

$$\gamma_R = R\phi / H \qquad (11)$$

$$\tau_R = 2\mathrm{M} / \pi R^3 \qquad (12)$$

where φ depicts the maximal deflection angle, H the plate gap width and M the measured maximal torque in the oscillation test. The characteristic storage and loss moduli G' and G'' could be calculated according to the following equations, using the phase-shift angle δ between applied strain (deformation) and measured shear stress function (Mezger, 2000):

$$G' = \tau_R / \gamma_R * \cos\delta \qquad (13)$$

$$G'' = \tau_R / \gamma_R * \sin\delta \qquad (14)$$

The effect of storage temperature and temperature fluctuations on the ice cream viscosity has not been studied.
On the other hand, the effect of storage temperature and its fluctuations on the most important quality parameters of ice cream, such as total viable counts, free fatty acids and peroxide value (PV), and sensory characteristics, has been studied. The temperature profiles included constant super-freezing conditions at -60ºC, common storage temperature of -30ºC and, fluctuating storage conditions which involved three cycles of -30ºC to -10ºC to -30ºC (for 48 hours each cycle) on consecutive weeks followed by storage at steady -30ºC. Regarding free fatty acids and PVs, no significant differences between the tested temperature regimes were observed at the first four weeks of storage. However, these values then increased and, as expected, they were higher for the fluctuating temperature regime. The total viable counts maintained within acceptable limits (2-4 log CFU/g) during the 8 weeks of storage with no significant differences between the tested temperature regimes. With regards sensory evaluation, super-freezing conditions maintained ice cream quality. In conclusion, super-freezing conditions seem to be beneficial for long-term storage, but the increased cost of such storage must also be taken into account (-60°C is not enviseageble storage conditions). Fluctuating temperature profiles below the freezing point seem to have an adverse effect on the quality of ice cream, promoting in most cases the development of rancidity. Thus, the need to store ice cream and related products at a constant of -30ºC or even colder is obvious (Gormley et al., 2002).

Flores A. A. and Goff. H.D. (1999) have shown that there is no effect on the overall ice crystal size of stabilized or unstabilized ice cream samples if stored under low constant temperature (–30°C); however clear evidence on sample microstructure was shown when stored at a higher temperature (–16°C). Another important effect was the frequency or time length of temperature cycles is increased and smaller crystals tends to disappear. The governing recrystallization mechanism at this stage would have most likely involved partial melting and refreezing of ice crystals. Figure 4 shows cryo-scanning electron micrographic images of ice cream after temperature fluctuations. The authors notice the tremendous increase in crystals size that has occurred in the product after heat shock (bottom picture), compared to fresh made ice cream (top pictures).

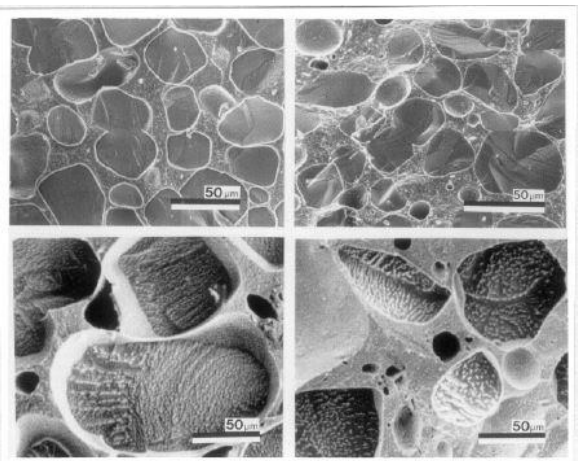

Figure 4. Cryo-scanning electron micrographic images of ice cream before and after temperature fluctuations from Flores A. A. Goff. H.D. (1999)

In the majority of the papers cited in the literature, it is reported that the more significant quality indicator for ice cream is ice recrystallization. The term recrystallization encompasses any change in number, size, shape, orientation or perfection of crystals following completion of initial solidification. Although ice recrystallization is of high significance for ice cream and dependent on storage temperature and temperature fluctuations (Flores A. and Goff H. D., 1999), the work reported is limited with most of it concerning modeling of data occurring from computer simulation and not from experimental data. One of these works is the paper of Ben-Yoseph and Hartel (1998) who modeled the ice recrystallization in ice cream during storage. The kinetics they used were developed from data obtained by Donhowe and Hartel (1996a) and reported by Donhowe and Hartel (1996b). Recrystallization during storage at different temperatures in vanilla ice cream with 40% total solids [12.0% (w/w) fat, 11.0% serum solids, 16.5% sucrose, 0.30% stabilizer (250 bloom gelatin), 0.1% emulsifiers (consist of 80% glycerol monostearate and 20% polysorbate SO)] was determined. The ice cream was subjected to accelerated recrystallization on a microscope cold stage.

The relationship between the population-based mean ice-crystal size, $\boldsymbol{L_{l,o}}$ (μm) obtained by image analysis, and the storage time, t (days) was (Donhowe and Hartel, 1996b),:

$$L_{1,o}=k\cdot t^{1/3} \qquad (15)$$

where k (μm/day$^{1/3}$) is the temperature-dependent recrystallization rate constant at any storage temperature.

The relationships between k and temperature were found using Arrhenius and William-Landel-Ferry (WLF) equations. Although the two regression results were very good, the WLF relationships were used in this simulation because it is more likely that these kinetics apply from the freezing point down to the glassy temperature (T_g). The WLF function is given in Eq. (16):

$$\log k = \log k_0 + \frac{\alpha(T-T_g)}{b+(T-T_g)} \qquad (16)$$

where α, b, and k_0 are constants.

The recrystallization rate was much higher for ice cream subjected to oscillating storage temperature than for constant temperature. The recrystallization rate constant for different amplitudes of fluctuation at mean temperature of -10°C gave small values of k at low amplitudes (0-1°C), a rapid increase in k at medium amplitudes (1-2.4˚C) and a small change in k at high amplitudes (2.4 - 4.0˚C). This is a typical sigmoid-shaped behavior and so a sigmoid shaped curve was used to describe the relationship between k and temperature amplitude:

$$k = \frac{c_1}{1+e^{c_2(\Delta T/2-c_3)}} + c_4 \qquad (17)$$

where ΔT is the temperature amplitude, and c_1, c_2, c_3 and c_4 are constants for specific temperature conditions.

Ben-Yoseph and R. W. Hartel, 1998 have shown the change in ice-crystal size distribution at different storage conditions: constant temperature of -5°C; and at mean temperature of -5°C with sinusoidal oscillations of + 1°C (6 h^{-1}). In addition, they reported the ice-crystal size at different storage temperatures after 72 h with no oscillations and with sinusoidal oscillations of ±1°C (6 h^{-l}) (Fig. 5).

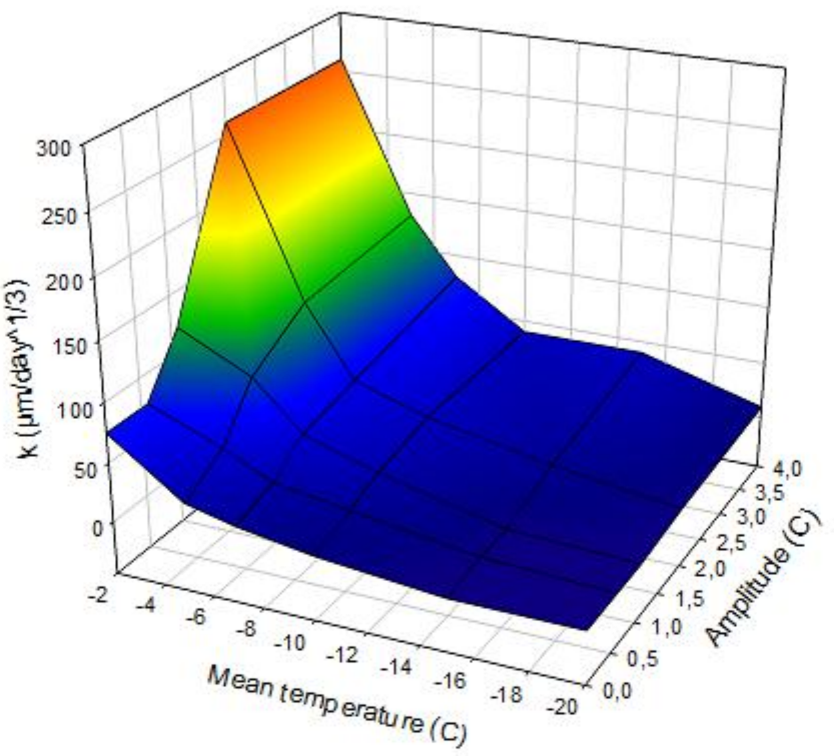

Figure 5. Effect of storage temperature and amplitude of temperature oscillation on ice recrystallization rate (k). Ben-Yoseph, R. W. Hartel 1998

During the distribution cycle the ice cream undergoes a series of temperature changes (Fig. 6). At the manufacturing plant, the mean storage temperature ranges from - 18°C to -30°C. At the distribution depot or central warehouse, the typical storage temperature is between - 23°C and -26°C. Ice cream is displayed in the supermarket at a wide range of temperatures that can range between -15.6°C and -2.8°C.. During transportation, the temperature of the ice cream can increase by 3-8°C (20°C according to the table) depending on the type of distribution vehicle. Finally, the temperature conditions in the home-freezer range from -12°C to -3°C. The storage duration at each of the above stages varies widely from a few days to more than 4 weeks.
Common mean temperatures, temperature fluctuations (as sinusoidal wave) and storage times were compiled from a variety of sources by Donhowe (1996). The conditions used in this study to simulate the storage and distribution system are given in Table 4.
The values for $L_{1,0}$ depend on the position in the container because the temperature is different at different locations. Average $L_{1,0}$ were calculated as the weighted averages of all $L_{1,0}$ in the container where a weight is a volume for specific values of $L_{1,0}$. These values are given in Table 4.
The average ice-crystal size in the container increased from 34.9 pm after the hardening process, to 46.1 pm at the end of the distribution cycle. The final value is slightly above the average of 43 pm as given by Berger and White (1979), and closer to threshold detectable size. The last column in Table 4 shows the average rate of change of $L_{1,0}$**.** From this column we can conclude that three factors have the most influence on the recrystallization rate: storage temperature in the plant, initial crystal size and storage duration. Large changes in $L_{1,0}$ during storage at the manufacturing plant were due to the initially small size of ice crystals which grew rapidly (small crystals are expected to be more sensitive to temperature fluctuation). The large changes during transporting in the consumer vehicle were due to the high surrounding temperature. The large changes during storage in the home freezer were due to the relatively long storage duration and high storage temperature and to the temperature fluctuation.

Table 4. Simulated Mean Ice-crystal Size (L,,cJ and Coefficient of Variance (c.v.) of Ice Cream During Typical Storage Conditions. Value Reported at the End of Each Storage Period

Storage site	Storage time	Mean/amplitude/rate of storage temperature	Average $L_{1,0}$ (μm)	Average c.v.	Change in $L^{1,0}$ per 1 day storage (μm/day)
Initial conditions of hardening	—	Ice-cream temperature = −18°C	35.0	0.45	—
Manufacturing plant	2 weeks	−22.0°C/2°C/6 h^{-1}	41.1	0.47	0.44
Distribution vehicle from plant	6 h	−19.0°C/2.8°C/6 h^{-1}	41.2	0.49	0.20
Central warehouse	4 weeks	−24.0°C/6°C/6 h^{-1}	42.1	0.50	0.04
Distribution vehicle from warehouse	3 h	−19°C/2.8°C/6h^{-1}	42.2	0.53	0.16
Supermarket storage	1 week	−15.6°C/2.8°C/6 h^{-1}	43.1	0.56	0.13
Consumer vehicle from supermarket	0.5 h	20°C/0°C/0 h^{-1}	43.1	0.56	0.96
Home freezer	1 week	−12°C/2.8°C/6 h^{-1}	46.1	0.58	0.43

(Ben-Yoseph, R. W. Hartel 1998)

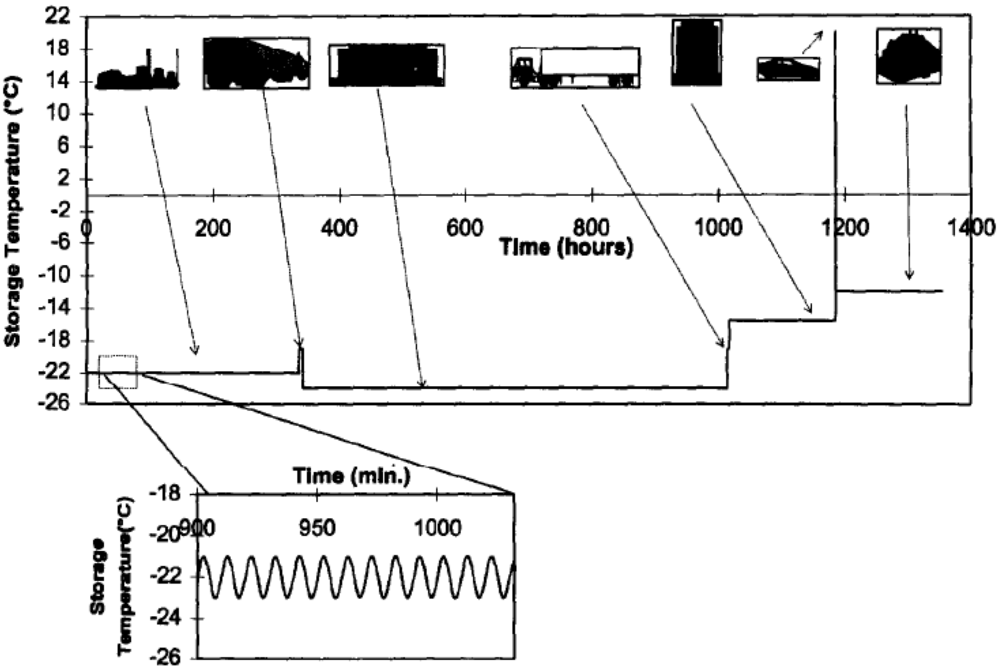

Figure 6: Temperature profile during typical distribution chain for ice cream. Ben-Yoseph, R. W. Hartel 1998

According to the simulation results, most of the recrystallization in ice cream occurs during the initial storage stages, which are under the control of the manufacturers. Storing the ice cream at constant low temperatures (-22°C) conditions at the manufacturing facilities is critical to maintaining ice-cream quality. The ice crystals are small but might grow very fast if temperatures increase. It is recommended that a cooling device with strong convection heat transfer is used to contribute in reducing temperature fluctuation as much as possible. Storage temperature in supermarket storage are relatively high compared to the recommended ones (at least -22°C) and amplitudes are important for longer shelf life, since significant coarsening can occur at this point if conditions are not controlled. A high rate of change in the crystal mean size has been found during the consumer transportation and domestic freezer storage periods.

In conclusion, ice cream is a product whose quality is highly correlated to the storage temperature and temperature fluctuations. There is limited data in the literature concerning modeling of the effect of temperature on texture, viscosity and ice recrystallization. NTUA will conduct experiments for the accomplishment of WP 3, studying the effect of static and dynamic storage temperature conditions (fluctuations of temperature is a significant factor to be studied). Texture, viscosity and ice recrystallization will be studied using appropriate equipment and methodology. The obtained data will be evaluated and modeled vs time and storage temperature. Equations will be developed to describe the effect of storage conditions on texture and viscosity parameters. For the corresponding description of ice recrystallization, the above described equations will be used. The validation of these models (concerning ice recrystallization) is of high significance since these equations have been assumed by simulating the cold chain of the ice cream. Real cold chain scenarios from WP2 collection of cold chain existing data will be used.

7. Conclusions

In conclusion, published work is available with regards modeling frozen spinach shelf-life. Vitamin C has been considered as the main deteriorative factor for this kind of product. Nevertheless, the spinach vitamin C retention has to be correlated with other quality indices such as colour, texture etc important to the consumers to evaluate the remaining shelf-life. Thus in the context of WP3, optimally designed storage experiments will be conducted at isothermal and dynamic conditions to validate the shelf life models under “real” distribution and storage conditions scenario based on data introduced in the database on cold chain in Europe developed in WP2. The already generated models will be used to describe the temperature effect on vitamin C degradation. These experiments besides vitamin C will include in parallel and correlate other important quality indices (colour, chlorophyll degradation, sensory scoring). Variability in factors that may affect vitamin C loss and shelf life, such as pH and moisture content of the product will be introduced in the equations. The models that will be developed will be applicable for frozen spinash products of varying characteristics and for the dymanic conditions of the cold chain.

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