EFFECT OF TEMPERATURE ABUSE ON FREEZE-THAW CHARACTERISTICS AND MICROBIAL QUALITY OF FROZEN ARMY RATIONS: A NUMERICAL STUDY

By

KARTHIKEYAN JAGADEESAN SANKARAN

A thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Food Science

written under the direction of

Professor Mukund V. Karwe

and approved by

________________________

________________________

________________________

New Brunswick, New Jersey

October 2013
ABSTRACT OF THE THESIS

EFFECT OF TEMPERATURE ABUSE ON FREEZE-THAW CHARACTERISTICS AND MICROBIAL QUALITY OF FROZEN ARMY RATIONS: A NUMERICAL STUDY

by J. S. KARTHIKEYAN

Thesis Director:
Professor Mukund V. Karwe

Frozen army rations for the deployed US army units are manufactured in the US and shipped to army bases in various countries. The transportation usually takes 3 to 5 months and the frozen rations are stored at -18 °C in large containers. Under ideal conditions, the frozen food items are expected to have a frozen shelf-life of 9 months. In reality, the food items might get exposed to severe temperature abuse during transportation, which may lead to microbial spoilage and/or quality loss. The aim of this study was to predict the effect of external temperature fluctuations on the temperature distribution and the microbial quality of frozen army rations during storage and transportation.

An army breakfast menu box containing five different food items, was selected for conducting this research. Commercial software COMSOL® Multiphysics was used to numerically predict the thermal behavior of the food items under varied external temperature conditions. Freeze-thaw properties of each food item were obtained using
component data and Differential Scanning Calorimetry (DSC), which were then used in
the numerical simulation of heat transfer with phase change. Apparent Specific Heat
(APH) method was used to account for the latent heat of a phase change during thawing
and refreezing. The predictive model was validated in terms of variation of temperature
with time at selected locations in a gel-based model food system and in a real food
system. Microbial quality was used as the primary parameter that determined the shelf-
life of food. ComBase database was used to identify the most prone microorganism and
to obtain its growth kinetics.

Microbial kinetics data was incorporated in the numerical simulation program to
predict the microbial quality of army rations during two temperature abuse scenarios: (i)
constant high external temperature exposure and (ii) freezer breakdown. Numerical
predictions suggested that the food items which was exposed to surrounding constant
temperatures of (20, 25, 30, 35, and 40) °C, can be allowed to stay at those temperatures
for a maximum time of (29, 20, 15, 12, and 9) h, respectively, and in the case of freezer
failure, the food items can be allowed to stay in the broken freezer for a maximum time
of 186 hours, to ensure food safety.
ACKNOWLEDGEMENTS

First and foremost, I would like to express my profound thanks to my advisor, Dr. Mukund V. Karwe for his excellent guidance, support and encouragement throughout my Masters. I am grateful for him for providing me the opportunity to work on this project. I am extremely fortunate to work under his guidance.

I would extend my deepest thanks to Dr. Kiran Desai, for sharing his valuable experience, suggestions and for his constant support throughout this project.

I would sincerely like to thank Dr. William Franke and Dr. Michael Rogers for agreeing to serve on my thesis committee. Their inputs, suggestions, and comments are appreciated.

I would like to thank, Natick Soldier Systems Center - U. S. Army for funding this project. I wish to thank Rieks Bruins, the PI of this project for his help and support. I would like to thank Dr. Donald Schaffner for his help in microbiological studies. I would also like to thank my project mate Neha Bhide for her input in chemical stability studies.

I would like to express my sincere appreciation to my lab mates, Swetha, Jose, Neha, Soundharya, Meenakshi, Li Zhang, Tanya, Siddhi, Rajay, Yijing, Siddharth, and Ji Lin for creating a fun and friendly environment and for their support in developing my personal skills. Special thanks to Soundharya for her support and help in proofreading my thesis.
I would like to thank my friends, near and far, for their encouragement and emotional support. I am also grateful to my family members for their support.

No words of mine can adequately express my regards and love to my respected parents, for their encouragement and support. Their help and moral support have always created confidence in me to accomplish my goals.

Finally, I pay my gratitude to the Almighty GOD for giving me strength and blessings.

Matha, pitha, guru, theivam and my friends. Thank you very much.
# TABLE OF CONTENTS

ABSTRACT OF THE THESIS.................................................................ii

ACKNOWLEDGEMENTS......................................................................iv

TABLE OF CONTENTS.......................................................................vi

LIST OF TABLES................................................................................x

LIST OF FIGURES..............................................................................xi

1. INTRODUCTION..............................................................................1
   1.1 Justification of the research.......................................................1
   1.2 Objectives of the research.........................................................2

2. BACKGROUND................................................................................4
   2.1 Frozen food..............................................................................4
      2.1.1 UGR-A transportation system...........................................5
   2.2 Freeze-thaw properties of food................................................6
      2.2.1 Fourier heat transfer equation.........................................7
      2.2.2 Mushy zone.....................................................................7
      2.2.3 Apparent Specific Heat (ASH) method..............................10
   2.3 Differential Scanning Calorimetry (DSC).................................12
   2.4 Numerical simulation...............................................................13
      2.4.1 Finite Element Method (FEM)...........................................15
2.5 Quality parameters ........................................................................................................15
  2.5.1 Quality kinetics .................................................................................................16
  2.5.2 Quality predictions .........................................................................................17
2.6 Conclusions of literature review ..............................................................................18

3. MATERIALS AND METHODS .................................................................................20
  3.1 Food description ..................................................................................................20
    3.1.1 Real food system - Menu box ....................................................................20
    3.1.2 Model food system .....................................................................................23
  3.2 Differential Scanning Calorimetry analysis .........................................................24
    3.2.1 Apparent specific heat ..............................................................................25
    3.2.2 Initial freezing/final thawing point ...............................................................25
    3.2.3 Latent heat of phase change .......................................................................26
    3.2.4 Bound water ................................................................................................27
    3.2.6 Unfrozen water fraction and Ice fraction ....................................................27
  3.3 Thermo-physical properties ...............................................................................28
    3.3.1 Density ........................................................................................................29
    3.3.2 Thermal conductivity ................................................................................29
    3.3.3 Apparent Specific Heat (ASH) ...............................................................30
  3.4 Mathematical modeling ......................................................................................33
    3.4.1 Geometry ....................................................................................................33
    3.4.2 Governing equations ................................................................................35
4.4 Microbial study ................................................................. 67

4.4.1 Microbial growth kinetics ............................................. 68

4.5 Temperature sensitive region ........................................... 70

4.6 Thawing time ................................................................. 71

4.7 Freezing time ................................................................. 76

4.8 Case studies ................................................................. 77

4.8.1 Case 1: Menu box exposed to high external temperature ... 77

4.8.2 Case 2: Freezer breakdown .......................................... 81

5. CONCLUSIONS ............................................................... 84

6. FUTURE WORK .............................................................. 86

7. REFERENCES ................................................................. 87

APPENDIX ........................................................................... 92
LIST OF TABLES

Table 3.1: Compositional data of the targeted food items in terms of mass fraction.......23

Table 3.2: Thermo-physical properties of individual food components.....................28

Table 3.3: Environmental real time temperature fluctuation data..........................50

Table 4.1: Latent heat of phase change and bound water fraction of food items..........57

Table 4.2: Maximum allowable time at each external temperature..........................80
LIST OF FIGURES

Figure 2.1: UGR-A transportation system: typical path of a menu box.........................5

Figure 2.2: Water and ice fractions in three different zones during phase transition of food..........................................................8

Figure 2.3: Apparent specific heat curve.........................................................11

Figure 3.1: (a) Beefsteak, (b) five beefsteaks packed in a box, (c) Orange juice, (d) Peppers & onions, (e) French toast, and (f) Danishes...............................21

Figure 3.2: Five different food items displayed in a box........................................22

Figure 3.3: Model food system - 25 % gelatin gel..............................................24

Figure 3.4: Heaviside function.................................................................31

Figure 3.5: 3D geometry of menu box........................................................33

Figure 3.6: (a) 3D geometry of cylindrical gel, in which radial section was used for simulation (b) 2D rectangle, axisymmetry at center..................34

Figure 3.7: Schematic diagram showing the mesh for the computational domain........39

Figure 3.8: (a) Top view of menu box with product distribution and thermocouple locations (b) Schematic representations of data acquisition system attached to the menu box.........................................................40

Figure 3.9: (a) Gelatin food system with four thermocouples inserted in to it (b) 3-D geometry of gel system (c) 2D radial section used for simulation - with four thermocouple locations.....................................42
Figure 4.1: DSC thermogram obtained during (a) freezing and (b) thawing for five different food items

Figure 4.2: (a) DSC thermogram obtained during freezing of two different types of gels

(b) DSC thermogram obtained during thawing of two different types of gels

Figure 4.3: Freezing experiment of beefsteak

Figure 4.4: Apparent specific heat curve for (a) freezing and (b) thawing processes of five different food items

Figure 4.5: Unfrozen water fraction as a function of temperature as a function of temperature during (a) freezing and (b) thawing

Figure 4.6: Experimental validation of frozen system at a specific location of

(a) 25% gel left in ambient environment

(b) 25% gel inside a insulated box

(c) 30% gel left in ambient environment

(d) 30% gel inside a insulated box

Figure 4.7: Experimental validation of frozen real food system at 4 known locations

(a) Beefsteak (b) Orange juice

(c) Peppers and onions (d) French toast

Figure 4.8: Experimental validation of freezing process of beefsteak
Figure 4.9: Maximum storage time as a function of product temperature - Influence of different microorganisms over the substrate resembling beefsteak........68

Figure 4.10: *Pseudomonas spp.* count increase at different temperatures - modeled using ComBase.................................................................69

Figure 4.11: Arrhenius model for *Pseudomonas spp.* growth kinetics....................70

Figure 4.12: (a) 3D geometry of menu box - Temperature sensitive region (b) 3D geometry of Beefsteak with the highlighted sensitive region.................71

Figure 4.13: 3D geometry of menu box with description........................................72

Figure 4.14: Temperature distribution within frozen menu box during thawing when exposed to 30 °C at different time intervals.................................72-73

Figure 4.15: Thawing time of all the food items when exposed to different constant external temperatures..................................................75

Figure 4.16: Relation between freezing time and product temperature....................76

Figure 4.17: Time-temperature history of most sensitive point in beefsteak..............78

Figure 4.18: Microbial log increase during exposure of menu box to different external temperature.................................................................79

Figure 4.19: The relation between external temperature and maximum allowed exposure time.................................................................81

Figure 4.20: Time-temperature profile for freezer breakdown scenario....................82

Figure 4.21: Microbial growth profile during freezer breakdown scenario..............83
1. INTRODUCTION

1.1 Justification of the research

Unitized Group Ration – A (UGR-A) is the most widely accepted military operational ration by the US army. These rations are manufactured in the US and shipped to the deployed US army bases around the world. A significant portion of the rations contains frozen food products, which are sorted and packed in boxes, according to different menus. The transportation usually takes 3 to 5 months and the boxes are supposed to be stored at -18 °C throughout the process. Under this ideal condition, the food items are expected to have a frozen shelf-life of 9 months. Since food safety and quality are largely dependent on the storage temperature, avoiding extreme temperature fluctuations will ensure frozen shelf-stability. Specifically, for frozen food items, significant temperature fluctuations above the freezing point may have detrimental effect on the microbial quality of food items (Reid, 1990; Rules, 1999). Therefore it is important to maintain the temperatures of the food items sufficiently below 0 °C.

In reality, temperature fluctuations are inevitable during transportation from US mainland to military bases in the Middle East and Far East, when different means of transportation are used, with unloading and loading of the food crates items. Understanding the impact of inadvertent temperature abuse of frozen food items is an important element in designing effective food safety controls. Temperature monitors are not helpful in predicting the safety of food at the consumption end because inferring the extent of spoilage and safety from the temperature history alone is difficult. Therefore, it
is imperative to have a technological solution for the UGR-A frozen boxes that would alert the end user about the temperature abuse the food had undergone. A numerical program that can successfully combine the temperature history of the food with the possible microbial kinetics has to be developed to monitor the food safety of frozen rations during transportation. Previously published papers provide information about heat transfer models for simple geometries and also for incorporating microbial kinetics with the heat transfer model. A detailed study on frozen foods, which includes phase change and its effect on the overall safety, has not been done. Thus, the scope of this project was to develop a heat transfer model for frozen food products based on their phase changing properties.

This research primarily focused on numerical simulation, because it is a more convenient and efficient method to simulate temperature distribution in frozen food products. Combining the numerical program with the microbial kinetics helps in food safety prediction. A Time-temperature relationship that would ensure food safety under possible temperature abuse scenarios was derived from the numerical simulation. Thus, the results of this research can be used to design a Time Temperature Integrator (TTI), which can indicate the extent of food safety to the end user by simpler visual means.

1.2 Objectives of the research

The main aim of this research was to quantify the effect of external temperature abuse on the microbial quality of a frozen army breakfast menu box containing beefsteak, orange juice concentrate, peppers & onions, French toast, and danishes. During the
temperature abuse scenario, based on the experimental data (not part of this thesis), chemical degradation of the food items was assumed to be negligible when compared to the microbial spoilage. Hence, microbial quality was chosen to define shelf-life of the food items. The specific objectives of the study were:

1. To carry out a three-dimensional numerical simulation of heat transfer for the menu box containing five different food items so as to predict the temperature distribution inside the box. Experimentally validate the numerical predictions in terms of temperature variation with time, at selected locations.

2. To evaluate the microbial growth kinetics of the most common spoilage/pathogenic microorganism that is prone to grow on the most sensitive food item in the menu box.

3. To combine the validated numerical simulation model for heat transfer with microbial growth kinetics to determine the food safety during two possible temperature fluctuation/abuse scenarios.
2. BACKGROUND

2.1 Frozen food

Freezing is one of the most prevalent and successful methods for food preservation to ensure better retention of food quality over time. Freezing and storage of food below 0 °C reduces microbial activity by delaying metabolic reactions. Freezing also reduces deteriorating chemical reaction rates by inhibiting enzyme activity and by lowering water activity. Hence, freezing ensures food safety with extended shelf-life and also minimizes changes in appearance, odor, and flavor (Singh and Wang, 1977). Biological changes are completely inhibited at very low temperatures of -80 °C. A wide range of sub-zero storage temperatures has been proven to be the best for various frozen food products. For commercialization, storage temperature of -18 °C or below is recommended for food products (Klose et al., 1959; Khan et al., 1963; Singh and Wang, 1977). Storage temperature of -18 °C cannot reduce the initial microbial load, but it will suppress the microbial growth. At that temperature, enzymatic and non-enzymatic reactions continue at very slow rates. To increase the consumption of frozen food, the shelf-life of the food items can be extended by temperature-controlled storage system called cold chain storage (Rules, 1999).

In reality, during cold chain transport, frozen food may encounter unintentional temperature abuse due to many reasons: freezer breakdown, extended stay at a warehouse outside the freezer etc., where food items might lose their quality and safety due to the freeze thaw cycle (Moureh and Derens, 2000; Blond and Le Meste, 2004). Elevation of
storage temperature or fluctuations in storage temperature tend to have harmful effects on food items by either promoting microbial growth or by increasing chemical reaction rates (Reid, 1990; Rules, 1999), both reduce the shelf-life considerably.

2.1.1 UGR-A transportation system

The frozen food items selected for this research were from the US army breakfast menu box containing five different food items. All the food items in their respective packages are arranged in a fixed orientation inside the menu box. Figure 2.1 shows the path of frozen food items during transportation from the US to a typical army base.

Figure 2.1: UGR-A transportation system: typical path of a menu box
The transportation time via road and via sea by different means takes about 3 to 5 months. It is imperative to maintain the storage temperature of -18 °C during the transportation, in the warehouse, and also during transit between two modes of transportation. Different modes of transportation with loading and unloading in between, increase the possibility of temperature abuse for frozen army rations.

### 2.2 Freeze-thaw properties of food

Analysis of freezing and thawing process belongs to a special branch of heat transfer called cryogenic heat transfer. Factors including phase change and temperature dependent thermal properties play an important role in cryogenic heat transfer, which are negligible in general heat transfer (Zhongjie et al., 2003). Freezing/thawing time and temperature profile within food can be determined experimentally or predicted approximately by either analytical or numerical methods (Lin, 1992). Experimental procedures are often too expensive, time consuming and may lack a generalized theoretical description of the process (Lin, 1992). By comparison, numerical methods based on theory, are more effective in analyzing actual situation (Lin, 1992). The physical changes of food during phase change have to be understood for proper prediction of its thermal behavior at different temperature conditions. Knowledge of the thermo-physical properties such as initial freezing point, unfrozen water fraction and ice fraction, specific heat and enthalpy, density, thermal conductivity etc., are required for the prediction of temperature within food (Matuda et al., 2011).
2.2.1 Fourier heat transfer equation

A basic Fourier equation for heat conduction was used to predict temperature distribution in a material:

\[ \rho c_p \frac{\partial T}{\partial t} = \nabla (k \nabla T) + S \]  

(2.1)

where \( \rho \) is density in kg/m\(^3\), \( C_p \) is specific heat capacity in J/(kg \cdot K), \( T \) is temperature in K, \( t \) is time in s, \( k \) is thermal conductivity in W/(m \cdot K) and \( S \) is a source term in W/m\(^3\) referring to distributed heat source or heat sink (Ayasoufi and Keith, 2003). Density, thermal conductivity and specific heat capacity are the major thermo-physical properties, that are required to solve this heat transfer equation and evaluate temperature variation over space and time. For a given food, these major thermo-physical properties can be obtained from composition-based prediction method, i.e., the properties can be calculated from the properties of the major components in food, namely, proteins, fats, carbohydrates, fiber, ash and water/ice (Andreasen, 2009).

2.2.2 Mushy zone

The simplest approach for solving the Fourier heat transfer equation is to consider the food system as a bulk of foamy material in which water and ice experience phase transition during a freeze-thaw process (Watzke et al., 2010). Pure chemicals undergo the phase change at a fixed temperature resulting in a distinct interface separating solid and liquid phases, e.g., freezing of water or rapid solidification of metals. When the phase change and the latent heat flow take place at a single temperature, it is known as the
Stefan problem (Pham, 2006). In contrast, for a multi-component substance such as food, the latent heat will be released or absorbed over a range of temperature due to the concentration of solutes (Franke, 2000). Unlike the Stefan problem, frozen foods do not exhibit a sharp liquid/solid interface. The phase transition temperature range that separates the thawed and frozen food is called as the 'mushy' zone, which is characterized as liquid water region with suspended solid ice (Bhattacharya et al., 2002).

**Figure 2.2: Water and ice fractions in three different zones during phase transition of food**

Figure 2.2 is a pictorial representation of behavior of water present in food during phase transition. In a completely thawed food, 100 % of moisture present will be in liquid state. During freezing (right to left), the initial freezing point ($T_1$) is the temperature at which crystallization begins. The initial freezing point is one of the basic parameters needed for the estimation of freezing time and it is often a function of the product
composition (Bhattacharya et al., 2002). As the temperature decreases below the initial freezing point \( T_1 \), the unfrozen water fraction in liquid state starts decreasing from 100 % and the ice fraction starts increasing from 0 %. These fractions change as a function of temperature in the mushy zone. Bound water content \( (x_b) \) or un-freezable water is the fraction of water that remains unfrozen in a product below a reference temperature \( (T_2) \). This fraction of water remains in liquid state by being entrapped in food matrix. In freezing research, the reference temperature \( (T_2) \) for food is assumed to be -30 °C. Below this reference temperature, the food is termed as completely frozen. The percentage of bound water present in the frozen food may vary from 0.5 % to 30 % of the total moisture content depending on the temperature and type of food (Park, 2008). When freezing below \( T_2 \), the unfrozen liquid water reaches the constant bound water fraction and ice fraction reaches its maximum value. The unfrozen water fraction \( (x_{unf}) \) and ice fraction\( (x_{ice}) \) are given by piecewise equations (2.2) and (2.3) respectively.

\[
x_{unf}(T) = \begin{cases} 
  x_b & T < T_2 \\
  f_{unf}(T) & T_2 < T < T_1 \\
  100 & T_1 < T 
\end{cases}
\]  
(2.2)

\[
x_{ice}(T) = \begin{cases} 
  x_w - x_b & T < T_2 \\
  f_{ice}(T) & T_2 < T < T_1 \\
  0 & T_1 < T 
\end{cases}
\]  
(2.3)

where \( x_w \) represents total moisture content of the food (Bhattacharya et al., 2002).
In the mushy zone, two of the three major thermo-physical properties: density and thermal conductivity, vary constantly due to the change in unfrozen water fraction and ice fraction and this variation can be described as a function of temperature by smooth non linear curves. These thermo physical properties can be assumed to be constant in completely thawed and frozen regions (Voller, 1997).

2.2.3 Apparent specific heat

Density and thermal conductivity can be expressed from the composition data of individual food as discussed in section 2.2.1. The challenge in solving the heat transfer equation (2.1) is inclusion of specific heat and latent heat of phase change over the phase transition temperature range. Two methods analyzed by Voller (1997), to solve the heat transfer equation for heterogeneous system with the incorporation of latent heat in transient mushy zone, were fixed grid method and moving grid method (Kumar and Panigrahi, 2009). The fixed grid method includes the latent heat in source term (equation (2.1)) and solves for each fixed node, element, or finite volume. But, source term is not suitable for most foods, because the latent heat is released over a wide range of temperature and is hard to distinguish from sensible heat. In the moving grid method, the object is divided into a frozen zone and unfrozen zone. During phase change, appropriate interface conditions are imposed on the phase changing front and allowed to move with it. Moving grid method can yield precise, non oscillating solutions for substances undergoing sharp phase change, where the equation (2.1) is solved separately for solid phase, liquid phase and moving phase changing front. However, it is less flexible in case
of food, because freezing front and thawing front are not clearly defined during gradual freezing/thawing process of food (Kumar and Panigrahi, 2009). Hence, the fixed grid method and moving grid method are not suitable for solving the phase change problem of food. For the latent heat method developed for multi-component food systems, a single energy balance equation is required for the entire domain consisting of coexisting solid, liquid and mushy zone. The most popular and reliable technique is Apparent Specific Heat (ASH) method (Pham, 2006; Kumar and Panigrahi, 2009). In this method, latent heat is merged with sensible heat to produce a specific heat curve with a large peak around the phase transition temperature range as shown in the Fig. 2.3.

![Figure 2.3: Apparent specific heat curve](image)

The original heat transfer equation (equation (2.1)) is modified as

$$\rho c_{p(app)} \frac{\partial T}{\partial t} = \nabla (k \nabla T)$$

(2.4)

where, $c_{p(app)}$ is apparent specific heat capacity in J/(kg · K). $c_{p(app)}$ was obtained by differentiating the enthalpy change of the food during phase change, which include both
latent heat and sensible heat with respect to temperature as explained in equation (2.5).

\[ c_{P(app)} = \frac{dH}{dT} \]  

\( c_{P(app)} \) includes the thermal effects of phase transition for each time step during mathematical modeling (Floury, 2006; Le Reverend, 2008). For a food product, these thermo-physical properties can be calculated using composition data and data from Differential Scanning Calorimetry (DSC) thermogram.

### 2.3 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is used to determine the thermodynamic properties and their variation with temperature and composition of food materials. DSC has been successfully used to analyze many food items that undergo phase change. Few of them include sweet potato (Fasina, 2005), ice cream (Cogne et al., 2003), bread dough (Lind, 1991), and frozen dough (Zhang et al., 2007). DSC calculates the amount of heat energy released or absorbed by a food sample during temperature change. It provides information regarding the energy released or absorbed in the form of latent heat during phase transition of food. This method is based on a differential heat flux measurement between a sample cell and an empty reference cell.

The advantages of DSC are rapid and simple measurements and the fact that from a single thermogram, valuable information such as specific enthalpy, apparent specific heat, latent heat of phase change, ice fraction and unfrozen water fraction can be obtained (Cogne et al., 2003; Hamdami et al., 2004; Le Reverend, 2008; Sebnem et al., 2007). The
sample size used for measurement is very small. In the case of a heterogeneous material, sampling should be done with great care, such that the analyzed small sample should well represent the investigated food (Matuda, 2011). At such small quantities, the moisture present in the sample gets saturated with the dissolved solids which could lead to supercooling because of which determining the initial freezing point from DSC thermogram can become difficult. Therefore an additional experiment is needed to determine the initial freezing point and correct the DSC thermogram accordingly (Wang and Kolbe, 1991).

DSC has been used by many researchers to evaluate apparent specific heat and other thermo-physical properties of food (Sebnem et al., 2007; Le Reverend, 2008; Hamdami et al., 2004). Different methods have been tried to extract the apparent specific heat curve as a function of temperature from DSC thermogram. They are cubic polynomial curve fitting (Sebnem et al., 2007; Matuda, 2011; Andrew et al., 1999), heaviside function (Groulx and Wilson, 2009) and derivative method in which latent heat of food was differentiated with respect to temperature (Hamdami et al., 2004). The thermo-physical properties evaluated from DSC are being used in numerical simulation to predict the thermal behavior of frozen food under varied external temperature conditions.

2.4 Numerical simulation

A mathematical model for freeze-thaw process is a system of partial differential equations that solve the heat transfer in all three zones: unfrozen, frozen, and mushy
region. Heat transfer, during a phase change, is a complex non-linear problem in mathematics. The analytical or the exact solution for this problem is impossible to predict due to its complexity. Therefore, an approximate analytical approach, numerical simulation method, and experimental method have to be applied (Lin, 1992). The challenging step in modeling phase changes of frozen foods is the description of non-linear thermal properties in time and space. Though phase transition has a discontinuous nature, it is macroscopically observed as a continuous phenomenon. Thus, the thermo physical properties can be described by an empirical or derived mathematical equations in all three regions using thermo-physical relationships (Martins and Silva, 2004).

The numerical solution of equation (2.4) can be obtained by discretizing the space domain to obtain a set of Ordinary Differential Equations (ODEs) by relating the nearby known temperatures, and then solving this set of ODEs. The most common methods used in solving this problem are Finite Difference Method (FDM), Finite Element Method (FEM), and Finite Volume Method (FVM). The widely used FDM has difficulty in dealing with the boundary condition for problems with complicated shape and with severe non-linear challenges. It also sometimes has weak stability and slow convergence. Due to the complexity during phase change, discretization can be achieved using either Finite Volume Method (FVM) or Finite Element Method (FEM) (Kumar and Panigrahi, 2009). In terms of accuracy, time interval for convergence, heat balance error and computing effort, it had been proven that FEM has an advantage over FVM in solving phase change problems. (Zhongjie, 2003; Pham, 1995; Pham, 2006).
2.4.1 Finite Element Method

Finite element method (FEM) approximates the physical domain to a mesh of finite elements with connecting points in space called nodes (Henwood and Bonet, 1996). For each discretized element, a linear relationship between node temperatures is used to solve the heat transfer equation (Braess, 1997). According to FEM, latent heat effects can be approximated by an equivalent heat capacity over the small temperature mushy range by invoking both the sensible heat and the latent heat (Franke, 2000). Intermediate states and the phase change region are determined from the equilibrium liquid volume fraction vs. temperature relationship, thereby avoiding the necessity for a phase change front tracking algorithm. Hence, the method is ideal for studying phase change in multi-component materials (Pham, 2006; Kumar and Panigrahi, 2009).

2.5 Quality parameters

As frozen food products may be exposed to unavoidable temperature fluctuations, quality factors determining food quality and safety such as color, flavor, nutrition and microbial level may change during storage. Possible deteriorating chemical changes during temperature abuse of frozen food are

1. Pigment discoloration and oxidative reactions of uncooked meat (Decker and Hutlin, 1992)

2. Protein degradation in protein rich sea foods (Sarma et al., 2000)

3. Lipid oxidation in the frozen high fat raw cooked meat products (Lee et al., 1997; Rhee, 1988)
4. Gluten network damage and deterioration of yeast in dough and baked products (Varriano-Marston et al., 1980; Bhattacharya et al., 2003; Barcenas et al., 2003)


Microbial spoilage is predominant in meats and meat based products when compared to other frozen food items experiencing temperature abuse. Possible microorganisms that can grow in meats can be divided in to pathogens such as *Listeria spp.*, *Salmonella spp.*, *Escherichia coli* O157:H7, *Clostridium botulinum*, *Staphylococcues aureus*, *Yersinia enterocolitica* and spoilage microorganisms such as *Pseudomonas spp.* (Bollman et al., 2001; Trakulchang and Kraft, 2006; Silvia et al., 2009; Escartin et al., 2000; Bajard et al., 1996). Different antioxidant and antimicrobial additives has been used by researchers to minimize undesirable changes in meat and other food products (Bollman, 2001; Tan and Shelef, 2002).

### 2.5.1 Quality kinetics

The degradation of quality under different temperatures with respect to time has been analyzed to evaluate its quality kinetics. Development of rancidity in meat has been studied using ThioBarbituric Acid Reactive substances (TBARs) which are released during lipid oxidation. For quality assessment of protein rich seafood, in addition to TBARs, Total Volatile Basic Nitrogen (TVB-N) has been used (Sharma, 2000; Lee et al., 1997; Rhee, 1988). TVBN is a byproduct of protein degradation. Analysis of TBARs and TVBN levels in food stored at different temperatures over a period of time is usually done to evaluate the chemical degradation kinetics.
For evaluating microbial kinetics several literature sources are available online. The two common databases for predictive food microbiology are Predictive Microbiology Information Portal (PMIP) and ComBase Initiative. PMIP was developed by the U.S. Department of Agriculture (USDA), Food Safety & Inspection Service (FSIS), based on food microbiology research conducted in the USA. It allows users to model microbial growth in a number of food products under several conditions (http://portal.arserrc.gov/). ComBase is a much larger database; it was developed by a collaboration between USDA, Food Standard Agency, Institute of Food Research - UK, and University of Tasmania Food Safety Center - Australia. It allows users to search food microbiology research results focused on growth and inactivation models based on food type, microorganism and environmental conditions (temperature, pH, water activity and salt concentration). Currently ComBase database contains 40,740 records, a much larger number than PMIP because ComBase is developed by an international collaboration (http://www.combase.cc/). By combining numerical simulation program with chemical degradation kinetics/predicted microbial kinetics, food quality/safety can be predicted.

2.5.2 Quality prediction

Studies that combined temperature history with quality kinetics to forecast the end quality of food have been dated back to 1944. Hicks (1944) proposed a mathematical model to predict the extent of chemical reaction with temperature varying regularly in sinusoidal and square wave patterns. This work was later further developed by a few researchers including Powers et al. (1965), Labuza (1979), and Nunes and Swartzel
Scott and Heldman (1990) assumed a limiting single quality deterioration process in their research and coupled the quality kinetics with temperature history for predicting the degradation of frozen food, which is subjected to step changes in storage temperature. They worked on one-dimensional and two-dimensional finite difference numerical methods. Zuritz and Singh (1985) developed 1-D and 2-D axisymmetrical finite element based model to predict the effect of varying boundary condition over the physical properties of food. Elvira et al. (1996) worked on simple 3-D rectangular object and studied the varying temperature field in frozen food with different physical properties, which were subjected to varying external temperature. Though many works have been done in past to combine quality parameters with the thermal behavior of food, considering current market requirement, these studies on frozen food are mostly in preliminary stage: either analyzing simple geometrical food or using known changes in external temperature conditions. There is still a necessity to develop a model that can predict the quality of complex 3-D geometrical food items with different thermal properties that can undergo unexpected temperature abuse conditions during storage and transportation for long durations.

2.6 Conclusions of literature review

Based on literature review, it is clear that the rise in the consumption of frozen food, unlike fresh food with shorter shelf-life, has increased the need to ensure the shelf stability of frozen food with desirable end quality to the customer. This makes it essential either to maintain the required storage temperature or to take suitable measures for
monitoring the quality changes during temperature abuse conditions. Previous research works are insufficient to cope with the demand of the present US army rations studies, in which, phase change properties of the food items have to be considered while predicting quality.

In this thesis research numerical simulation of heat transfer for a menu box of frozen army ration containing five different food items, was carried out. It was combined with microbial kinetics to quantify the effect of surrounding temperature fluctuations on food microbial quality. The influence of packaging material properties, the orientation of food items inside the box, the influence of thermal behavior of other food items in the box, and the influence of the amount of headspace available for the food product, were investigated.
3. MATERIALS AND METHODS

The research primarily comprised of numerical simulation of heat transfer with some experimental investigation. The temperature history obtained from the experiments was used to validate the numerical predictions. The validated numerical algorithm was further combined with microbial kinetics so as to evaluate the effect of surrounding temperature abuse conditions over the safety of frozen food items.

3.1 Food description

Heat transfer simulation program was developed for a typical US army breakfast menu box containing five different food items. To verify the numerical prediction, experimental investigation was carried out using a real food system (menu box with five different food) and a model food system (gelatin based system).

3.1.1 Real food system - The Menu box

Dimensions of cardboard menu box were 49.5 cm × 39.4 cm × 30.2 cm. The description of food items packed in the menu box is as follows:

**Beefsteaks:** Five uncooked beefsteaks, each measuring 24.1 cm × 17.8 cm × 3.6 cm were individually packed (see Fig. 3.1(a)). All steaks were kept inside a cardboard box of dimension 39.4 cm × 27.9 cm ×14 cm (Fig. 3.1(b)).

**Orange juice:** Three cartons of concentrated orange juice, each measured 7 cm × 7 cm × 18.5 cm (Fig. 3.1(c)).

**Peppers & onions:** Two separate packs of peppers & onions with approximate dimensions of 27 cm × 15 cm × 5.6 cm (Fig. 3.1(d)).
Figure 3.1: (a) Individually packed uncooked beefsteak, (b) five beefsteaks packed in a box, (c) Orange juice, (d) Peppers & onions, (e) French toast, and (f) Danishes
**French toast:** Twenty four individual cylindrical French toasts of length 15.2 cm and diameter 2.5 cm each were packed in six different packages, four toasts in each. They were arranged inside a cardboard box of 31 cm $\times$ 20 cm $\times$ 13.5 cm (Fig. 3.1(e)).

**Mini Danishes:** Twenty four individual mini danishes of diameter 7.6 cm and height 1.9 cm were arranged in a tray of dimension 27 cm $\times$ 19.6 cm $\times$ 4.5 cm. Two similar trays were present in the menu box (Fig. 3.1(f)).

Ingredient lists of all the food items are provided in the Appendix section. These food items were manufactured in the US and were frozen at -18 °C. All the food items were arranged in the menu box in a particular orientation. Figure 3.2 displays all the food items.

![Figure 3.2: Five different food items displayed in a box](image-url)
The composition of each food item, i.e., proteins, carbohydrates, fats, fiber was obtained from their respective nutrition labels. Moisture content of the homogenized food items was measured in triplicate using Sartorius moisture analyzer MA 30. Since ash content in food is very low, it was assumed that each food item had approximately 1% ash (Andreasen, 2009). Compositional data of the targeted food items are given below in Table 3.1.

**Table 3.1: Compositional data of the targeted food items in terms of mass fraction**

<table>
<thead>
<tr>
<th></th>
<th>Fats</th>
<th>Carbohydrates</th>
<th>Proteins</th>
<th>Fiber</th>
<th>Ash</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beefsteak</strong></td>
<td>0.142</td>
<td>0</td>
<td>0.196</td>
<td>0.025</td>
<td>0.01</td>
<td>0.627</td>
</tr>
<tr>
<td><strong>Orange juice</strong></td>
<td>0</td>
<td>0.3662</td>
<td>0.0126</td>
<td>0.0012</td>
<td>0.01</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Peppers and onions</strong></td>
<td>0.0235</td>
<td>0.0941</td>
<td>0.012</td>
<td>0.0104</td>
<td>0.01</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>French toast</strong></td>
<td>0.17</td>
<td>0.41</td>
<td>0.06</td>
<td>0.01</td>
<td>0.01</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Danishes</strong></td>
<td>0.1714</td>
<td>0.5429</td>
<td>0.057</td>
<td>0.0067</td>
<td>0.01</td>
<td>0.212</td>
</tr>
</tbody>
</table>

**3.1.2 Model food system**

A model food system was used for validating the simulated results. Unlike a heterogeneous real food, the selected model food system was homogenous. So in addition to real food system, experiments with the model food system were conducted for validation. Gelatin was used to create model food system because it is freeze thaw stable.
No. G-2500 Gelatin from Sigma Aldrich was used for these experiments. Experiments with two different moisture contents: 70 % and 75 % (w.b.) of gelatin were carried out to investigate the effect of different moisture content on the thermal behavior of the food system. Figure 3.3 shows 25 % gelatin (w.b.) system. The gels were made in cylindrical shape of height 2 cm and diameter 4 cm. Compositional data were obtained from the nutritional label. Protein composition of gelatin was 99 % (d.b.), and all the other components were in negligible range. Remaining 1 % (d.b.) was assumed to be ash (Andreasen, 2009).

![Image of gelatin gel](image)

**Figure 3.3: Model food system - 25 % gelatin gel**

### 3.2 Differential Scanning Calorimetry analysis

Mettler-Toledo DSC instrument was used to analyze the enthalpy change of the food items during temperature change. DSC was carried out for five real food products and two model food systems. Each food item was homogenized in a mixer (Wary laboratory mixer) and a sample weighing around 10 mg was placed in a ME-27331
aluminum crucible for the DSC experiment. The temperature change rate was set at 10 K/min (Le Reverend et al., 2008). The samples were analyzed during freezing and thawing in the temperature range of -30 °C to +30 °C. The rate of change of enthalpy with respect to time/temperature graphs (thermogram) was obtained for all the food items. This experiment was done in triplicate. Matlab® (Version 2010a, Mathworks Inc., Natick, MA), was used to analyze the thermograms and evaluate the thermo-physical properties. Evaluation of each parameter is explained below.

3.2.1 Apparent Specific Heat (ASH)

DSC generated thermogram shows variation of heat flow rate expressed as W/g as a function of temperature in K. Based on equation (2.5), the enthalpy value in W/g at each temperature was divided by the temperature change rate 10 K/s to obtain Apparent Specific Heat (ASH) capacity (J/(kg · K)) as a function of temperature, as explained in equation (3.1) (Floury et al., 2006).

\[
C_{p(app)} = \frac{dH}{dT} = \left| \frac{dH}{dt} \right| = \frac{\text{Heat flow rate (W/kg)}}{\text{Temperature changing rate (K/s)}} = \frac{J}{kg \cdot K}
\]  

(3.1)

3.2.2 Initial freezing/final thawing point

Initial freezing point is the temperature at which freezing begins. This is the temperature at which the phase change starts during freezing process and the ASH graph has its maximum value. When the food is completely thawed or completely frozen, the composition of food remains unchanged, hence food exhibits constant ASH value in these
regions. During freezing, at initial freezing point, the ASH value immediately increases to a maximum value and drops slowly as the freezing process continues.

During thawing, final thawing point is the temperature at which the phase change completes and the food can be termed as completely thawed. ASH value will be constant in completely frozen state of food, and rises slowly once phase change begins. It reaches the maximum value at final thawing point and drops immediately to a constant value indicating the behavior of thawed food.

As discussed in section 2.4, DSC curves will have unusual initial freezing point due to small sampling during experiment. This deviation was corrected by shifting the freezing curves to its actual initial freezing temperature which could be obtained experimentally. To that purpose, experiments with real food samples were carried out. Thawed food items were frozen at -18 °C and their freezing curves were obtained by monitoring temperature using a ‘T’ type thermocouple inserted in them. The temperature at which curve becomes plateau denotes the region of phase change and this is the initial freezing point of that particular food item (Foong et al., 2010). DSC freezing curves of all the food items were shifted according to the experimentally obtained initial freezing points.

### 3.2.3 Latent heat of phase change

Latent heat of phase change is the amount of energy absorbed or released during phase change. This value is directly proportional to the water content in the food that undergoes phase change. The latent heat of phase change for a food item was calculated
by evaluating the area under the curve of the ASH curve during phase change. The value was expressed in J/kg. Latent heat of phase change was assumed to be a constant for a particular food item. Change of latent heat values during repeated freezing and thawing cycles was ignored in this study, as those many change of processes were not expected in this study.

3.2.4 Bound water

Bound water is the portion of water in frozen food that never freezes. The water is bound to the food matrix and does not undergo phase change. Equation (3.2) was used to evaluate the bound water fraction (Hamdami et al., 2004).

\[
\text{Bound water fraction} = \text{Total moisture content} - \frac{\text{Area under the curve}}{L_o} \quad (3.2)
\]

where \(L_o\) is the latent heat of fusion of water at 0 °C, 333 kJ/kg.

3.2.5 Unfrozen water fraction and ice fraction

In the mushy zone, the unfrozen water fraction changes as a non-linear function of temperature and this function was derived from the ASH curve. For thawing and freezing processes, the unfrozen water fraction as a function of temperature was obtained separately. At the initial freezing point or final thawing point, the unfrozen water fraction will be 100 %, i.e., unfrozen water fraction equals total moisture content of food. During phase change, unfrozen water fraction reduces and follows the trend of DSC ASH curve depending on the process. At the reference temperature -30 °C, the fraction reaches a constant value of bound water fraction, indicating completely frozen state. Ice fraction is
the complement of unfrozen water fraction. The remaining portion of the total moisture content, excluding unfrozen water fraction will be ice fraction. Matlab® was used to fit a cubic polynomial function to the non linear curve of unfrozen water fraction and ice fraction in the mushy zone as a function of temperature. As explained in section 2.2.2, piecewise equations for the unfrozen water fraction and the ice fraction were developed for the three regions: thawed (liquid), mushy zone, and frozen (solid).

3.3 Thermo-physical properties

The three major thermo-physical properties to be evaluated are density, thermal conductivity, and apparent specific heat. As discussed in section 2.2.3, the density and the thermal conductivity were expressed as function of temperature, based on the combination of the major constituents of individual food items. Thermo-physical properties of all the components are listed in Table 3.2.

**Table 3.2: Thermo physical properties of individual food components**

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Carbohydrates</th>
<th>Protein</th>
<th>Ash</th>
<th>Fiber</th>
<th>Water</th>
<th>Ice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Density (kg/m³)</strong></td>
<td>925.5</td>
<td>1599.1</td>
<td>1329.9</td>
<td>2423.8</td>
<td>1311.5</td>
<td>997.18</td>
<td>916.89</td>
</tr>
<tr>
<td><strong>Thermal conductivity (W/(m · K))</strong></td>
<td>0.1807</td>
<td>0.2014</td>
<td>0.1788</td>
<td>0.3296</td>
<td>0.1833</td>
<td>0.5711</td>
<td>2.2196</td>
</tr>
<tr>
<td><strong>Specific heat capacity (J/(kg · K))</strong></td>
<td>1984.2</td>
<td>1548.8</td>
<td>2008.2</td>
<td>1092.6</td>
<td>1845.9</td>
<td>4176.2</td>
<td>2062.3</td>
</tr>
</tbody>
</table>

(Watske, 2010; Kumar and Panigrahi, 2009)
For completely thawed food, when the temperature is above initial freezing point or final thawing point, moisture content was considered as 100% water. And in completely frozen food, bound water percentage was considered to possess properties of water and remaining fraction had property of ice. In mushy zone the water and ice fraction changed as a nonlinear function of temperature and their properties were evaluated accordingly.

3.3.1 Density

Density was calculated as a function of temperature from the component data using equation (3.3) (Watzke et al., 2010). Mass fraction of each component given in Table 3.1 and their respective density values given in Table 3.2 were used to obtain the density of food items as a function of temperature.

\[
\rho(T) = \frac{1}{\frac{x_i}{\rho_i} + \frac{x_{\text{unf}}(T)}{\rho_w} + \frac{x_{\text{ice}}(T)}{\rho_{\text{ice}}}}
\]  

(3.3)

where \(i\) denotes the dry components, namely, fats, carbohydrates, proteins, ash, fiber. \(x_{\text{unf}}\) is mass fraction of unfrozen water and \(x_{\text{ice}}\) is mass fraction of ice. In completely frozen and thawed state the value of density was expected to be constant and in mushy zone density will be expressed as a non linear function of temperature.

3.3.2 Thermal conductivity

Thermal conductivity as a function of temperature was calculated from the component data using equation (3.4). Volume fraction '\(X_c\)' of each component, needed
for the equation was obtained by equation (3.5) using respective mass fraction ‘x’ value (Table 3.1) and density value (Table 3.2) (Kumar and Panigrahi, 2009).

\[ k(T) = k_i X_i + k_w X_{unf}(T) + k_{ice} X_{ice}(T) \]  

(3.4)

\[ X_c = \frac{x_c/\rho_c}{\sum \left( \frac{x_c/\rho_c}{\rho_c} \right)} \]  

(3.5)

where i includes dry components. ‘X_{unf}’ is volume fraction of unfrozen water and ‘X_{ice}’ is volume fraction of ice.

### 3.3.3 Apparent specific heat capacity

Three different methods were used to extract an equation from the ASH curve of DSC thermogram. Aim of trying these methods was to compare their efficiency in developing a model closer to the reality. The three methods are explained below.

(i) **Cubic polynomial fit**

Matlab® (Version 2010a, Mathworks Inc., Natick, MA) was used to fit a cubic polynomial function to the ASH curve. Polynomial function is of order three. General form of cubic polynomial equation used for ASH, as a function of temperature is given in equation (3.6) (Sebnem et al., 2007; Matuda, 2011; Andrew et al., 1999).

\[ C_{p(app)}(T) = a(T)^3 + b(T)^2 + c(T) + d \]  

(3.6)

The cubic polynomial equation was considered only for the mushy zone. For the
completely thawed and frozen region, respective constant values of ASH obtained from the DSC were used (Sebnem et al., 2007; Matuda, 2011; Andrew et al., 1999). The piecewise equation is given as

\[
c_{p(app)} = \begin{cases} 
  c_{p(thawed)} & T < T_1 \\
  c_{p(cubic polynomial)} & T_1 > T > T_2 \\
  c_{p(frozen)} & T > T_2 
\end{cases}
\]

(3.7)

where \( T_1 \) is initial freezing point or final thawing point and \( T_2 \) is reference temperature -30 °C (Fig. 2.2)

(ii) Heaviside function

In mathematics, a heaviside function, also called as step function, is generally used to define piecewise function. An example of a heaviside step function is shown in Fig. 3.4.

![Figure 3.4: Heaviside function](image)
The ASH curve was regenerated as a heaviside function and was used to define food property (Groulx and Wilson, 2009). In Fig. 3.4, \( T_1 \) is initial freezing point or final thawing point. The heaviside function was developed such that the area under the curve created by heaviside function approximately equals the area under the curve of ASH curve.

**(iii) Derivative method**

Saad and Scott (1996) expressed the specific heat as the combination of individual component of each food. Mass fraction of individual component (Table 3.1) was multiplied with the respective thermal properties (Table 3.2) as shown in equation (3.8). Latent heat during phase change was incorporated in to this equation by a differential term. In the mushy region, the last term differentiates unfrozen water fraction with respect to temperature. Multiplying it with latent heat of fusion of water \((L_o)\) gives the amount of latent heat exchanged at that temperature. In solid and liquid region the differential term gets neglected automatically (Hamdami et al., 2004).

\[
C_{P_{app}}(T) = C_{p_i}x_i + C_{p_w}x_{unf}(T) + C_{p_{ice}}x_{ice}(T) + L_o \frac{dx_{unf}(T)}{dT}
\tag{3.8}
\]

where \(i\) includes, dry components, namely, fats, carbohydrates, proteins, ash, fiber. \(x_{unf}\) is mass fraction of unfrozen water and \(x_{ice}\) is mass fraction of ice, \(L_o\) is 333 kJ/kg.

Accuracy of these three methods in predicting thermal behavior of food was evaluated from the results obtained. The best method was used for further research.
3.4 Mathematical modeling

A finite element based commercial computational software, COMSOL® Multiphysics (Version 4.2, COMSOL Inc., Burlington, MA), was used to numerically predict the temperature distribution in frozen UGR-A menu box during temperature abuse conditions.

3.4.1 Geometry

For the real food system, a 3-D geometry, depicting the menu box with five different food items of known dimensions with the fixed orientation, was developed. Figure 3.5 represents the 3-D geometry developed in COMSOL® Multiphysics, with the description of individual food item.

![Figure 3.5: 3D geometry of menu box](image-url)
The geometry was simplified to minimize the complications related with convergence of equation and computational time. Individual beefsteaks, peppers and onions packs and orange juice cartons were assumed to be rectangular boxes. Twenty four individual cylindrical French toasts were considered as one big rectangular box of equivalent total volume, with the respective dimensions constituted by individual pieces. Similarly twenty four mini danishes were simplified to one rectangular box.

In case of the model food system, the gel sample in cylindrical shape was kept on its face with its axis in vertical direction. It was assumed that the temperature distribution was axisymmetric. Therefore, the numerical simulation could be carried out in a radial slice (shaded) of the cylinder (Fig. 3.6).

![Figure 3.6](image)

**Figure 3.6:** (a) 3D geometry of cylindrical gel, in which radial section was used for simulation  (b) Computational domain for numerical simulation.
Heat transfer was majorly through solid food. In addition, the velocity field in the headspace induced by the natural convection was included in the numerical simulation. The air gap in the head space of beef steak box, French toast box and danishes boxes were so small, so that the air there was assumed to be stagnant. Natural convective flow was considered only for the headspace of the bigger menu box and the velocity field was assumed to be laminar. Therefore the model was developed in COMSOL® Multiphysics through *conjugate heat transfer model* accounting for *laminar flow*.

### 3.4.2 Governing equations

Heat transfer model developed by COMSOL® Multiphysics was based on the equation (3.9).

\[
\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + Q
\]  

(3.9)

This equation includes the material property namely specific heat capacity \( C_p \), density \( \rho \), and thermal conductivity \( k \). \( Q \) refers to additional heat source, so it was neglected in our case because latent heat was included in the apparent heat capacity. The thermo-physical properties obtained from DSC were used as the input to solve the equation (3.9).

For defining the ASH as a heaviside function, an analytical function in COMSOL® Multiphysics called 'flc2hs' function was used. For defining the thermal properties of air in the head space, built-in material library of COMSOL® Multiphysics was used.
For laminar flow of air due to natural convection, the condition of wall in the internal sides of box is similar to the single-phase flow settings. To maintain the continuity of temperature in the internal wall that separates the fluid domain and the solid domain, the wall was defined by the equation (3.10).

\[ \mathbf{u} = 0 \]  \hspace{1cm} (3.10)

The natural convective airflow due to temperature difference was incorporated as heat transfer in fluid, solved using the Navier-Stokes equation (3.11) for incompressible fluid. The equation used was

\[
\rho \frac{\partial \mathbf{u}}{\partial t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \left[ -p + \mu (\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \right] + \mathbf{F} \]  \hspace{1cm} (3.11)

The physical meaning this momentum balance equation is

\[
\begin{align*}
\text{Accumulation of momentum per unit volume} &+ \text{rate of momentum}= - \text{pressure forces} + \text{shear stress} + \text{gravity forces} \\
&\hspace{1cm} (3.12)
\end{align*}
\]

In equation (3.11), \( \rho \) is density in kg/m\(^3\), \( \mathbf{u} \) is the velocity of air in m/s, \( p \) is pressure in Pa, \( \mu \) is dynamic viscosity in kg/(m \cdot s), and \( \mathbf{F} \) is the volume force term expressed in N/m\(^3\). The 'F' term signifies the flow of air due to gravity. To solve this equation, the acceleration due to gravity of 9.81 m/s\(^2\) with respect to the density of the air was assumed to be in the negative z direction, where z axis is in the direction of height of the box. This equation was solved only for the headspace of the menu box.
Boundary conditions

On the outer layer of the box which was exposed to the external temperature fluctuation, the boundary condition was defined by the following heat transfer equation (3.13).

\[-n \cdot (-k \nabla T) = h \cdot (T_{\text{ext}} - T)\]  

(3.13)

Two important terms are external temperature $T_{\text{ext}}$ in K and convective heat transfer coefficient $h$ in W/(m$^2 \cdot$ K). For the simulation, the value of heat transfer coefficient used was obtained from experimental investigation. This is explained in experimental validation section.

Packaging property

Each food item was individually packed. Incorporating all of their packaging thicknesses and their thermal properties in the 3-D geometry would have increased the number of elements leading to computational complications. To overcome this challenge, boundary condition thin thermally resistive layer available in COMSOL$^\text{®}$ Multiphysics was used. Thermal conductivity of the packaging material and its thickness were defined for each food item, so that equations (3.14) and (3.15) were solved accounting for the resistance provided by the packaging material to the heat flow.

\[-n_i \cdot (-k_i \nabla T_i) = -k \left( \frac{T_o - T_i}{d} \right)\]  

(3.14)
\[-n_o \cdot (- k_o \nabla T_o) = -k \frac{(T_i - T_o)}{d}\]  

(3.15)

where \(o\) and \(i\) refer to the outside and inside of the resistive layer. Here, \(k\) is the thermal conductivity in W/(m \(\cdot\) K) and \(d\) is the thickness in m, of the packaging material. The rate of heat flow across the outer and the inner sides of the packaging material will be the same. Based on this, equations (3.14) and (3.15) were equated and solved, to obtain the temperature drop due to the resistance provided by the packaging material. Individual beefsteaks, peppers and onion packs, French toast and one side of danishes box were packed in Expanded PolyStyrene (EPS), all the other boxes and orange juice cartons were packed in cardboard box. The thermal conductivity of EPS was 0.04 W/(m \(\cdot\) K) and that of the cardboard box was 0.065 W/(m \(\cdot\) K) (Kumar and Panigrahi, 2009). The thickness of all the packaging materials were in the range of 0.5 mm to 1 cm.

3.4.3 Mesh and Time step

Computational mesh was created using built-in mesh generating code in COMSOL® Multiphysics. In the 2D geometry, mesh was generated using triangular elements connecting 3 nodes at a time and for the 3D geometry, the elements were tetrahedral in shape connecting four nodes at a time. The effect of mesh size and time step on the final numerical solution was tested by successively refining the mesh, until negligible changes in the solutions were observed. Distance between each node varied from 4.95 mm to 3.96 cm depending on the complexity of geometry. For a typical menu box, the computational domain was discretized into approximately 20,000 elements as
shown in Fig. 3.7. The governing equations mentioned in the previous section were solved for these finite elements at appropriate nodes with the time step of 1 s. In addition, to indicate the convergence of the solution, convergence criteria was defined, in which relative tolerance was set to 0.01 and absolute tolerance was set to 0.001. Computational time needed for running a typical simulation case was about 40 hours on a Dell® workstation with Intel® Xeon® E5640 processor and 24 GB RAM.

Figure 3.7: Schematic diagram showing the mesh for the computational domain of menu box containing five different food items

3.5 Experimental validation

The numerically simulated heat transfer model was validated by experiments before simulating temperature abuse scenarios. Three sets of experiments used for validation were (i) thawing experiment with real menu box (ii) thawing experiment with gelatin food system and (iii) freezing experiment with individual real food items.
Experimental validation was also used to evaluate the heat transfer coefficient imposed by the external ambient condition on the outside of the box. For constant external temperature condition, the product temperature depends on the heat transfer coefficient. In the simulation, the heat transfer coefficient value was varied to obtain required product temperature after a given time.

3.5.1 Thawing experiment with real menu box

All five food items were arranged in a fixed orientation inside the menu box as shown in Fig. 3.8a. The simulation and experiment was done for this specific orientation. Four 'T' type Omega hypodermic needle probes were inserted at known locations of four items viz., beefsteaks, orange juice, peppers & onions and French toast (Fig. 3.8a).

Figure 3.8: a) Top view of menu box with product distribution and thermocouple locations (shown by red elipses)
Figure 3.8: b) Schematic representation of data acquisition system attached to the menu box.

Due to limited number channels available in the Data Acquisition (DAQ) system, the temperature variation was measured only at the four locations. Since danishes had the least amount of moisture content, temperature variation with time was not measured in danishes. The entire box was frozen at -18 °C to begin with. The frozen box was exposed to ambient air of 24 °C in a laboratory (no draft of air). While thawing the temperature variations with time at the four known points (Fig. 3.8b) as a function of time were recorded using a Data Acquisition (DAQ) system from National Instruments (LabVIEW™ 7 Express), Austin, TX. Using the DAQ system the temperature was recorded for every 3 seconds and the experiment was carried out for 48 hours.
3.5.2 Thawing experiment with model food system

Similar to previous experiment, four 'T' type Omega hypodermic needle probes were inserted along the axis of cylindrical gelatin based model food system (Fig. 3.9a). Experiments were done in both 25 % and 30 % gelatin system.

Figure 3.9: a) Gelatin food system with four thermocouples inserted in to it

Figure 3.9: b) 3-D geometry of gel system c) 2D radial section used for simulation - with four thermocouple locations
The gels were frozen at -18 °C. Frozen gels were then thawed at two different ambient temperature conditions: i) thawing at 24 °C, and ii) thawing inside an insulated box. The thawing in an insulated box experiment was carried out to verify the temperature gradient imposed by natural convection inside the headspace of the box. While thawing, the temperature at the four axial points as a function of time was recorded using the Data Acquisition system. Four thermocouple locations are mentioned and numbered in COMSOL® Multiphysics geometries (Fig. 3.9b and Fig. 3.9c).

### 3.5.3 Freezing experiment

As discussed in section 3.2.2, the thermograms generated by DSC during freezing process were shifted to the corrected initial freezing point obtained from freezing experiments. The simulation program developed with the corrected freezing curves was validated experimentally. For each food item, a 'T' type thermocouple was inserted at a known location and the temperature was monitored while the food was freezing at -18 °C. The experimental temperature history was compared with that predicted by the numerical simulation program.

### 3.6 Safety prediction

Meat products are most susceptible to microbial and chemical spoilage, so beefsteaks in the menu box were targeted for the safety/quality study. Information from the nutritional label indicated the presence of few antioxidants and preservatives in the beefsteaks, which can greatly reduce the possibility of chemical degradation. Generally chemical degradation takes place at a slower rate in frozen foods. In addition, the
presence of these additives is expected to reduce the reaction rate further. So in this study, chemical degradation was assumed to be negligible and the products were assumed to be chemically stable in frozen condition. At thawed condition, microbial spoilage rate in food will be much higher and relevant than chemical degradation rate. Thus, only microbial growth was considered as the parameter for quality and safety evaluation during the temperature abuse scenarios.

3.6.1 Microbial study

ComBase modeling toolbox was used to study the microbial growth. Most prominent microorganisms prone to grow in uncooked beefsteak were divided into pathogenic organisms such as *Escherichia coli* O157 H7, *Salmonellae*, *Listeria monocytogenes*, *Clostridium botulinum* and spoilage bacteria *Pseudomonas spp.* (Bajard, 1996; Bollman, 2001; Juneja et al., 2009; Trakulchang and Kraft, 2006; Peck et al., 2006). Exaggerated assumptions were made related to the microbial count, in order to minimize risks. The initial microbial count was assumed to be $10^5$ (CFU/ml). For pathogens the maximum allowable growth was assumed to be 1 log increase and for spoilage microorganism the spoilage criterion was assumed to be 2.5 log increase (Uyttendaele et al., 2001; Zhang et al., 2011; Ingham et al., 2007; Peck et al., 2006). Possibility of spore formation was not considered in this study. Substrate conditions similar to beefsteak were assumed, pH was varied between 6.5 to 7 and NaCl concentration was considered to be in the range of 0.5 - 1.5 %. Initial lag phase of microorganism was considered while predicting their growth level. With these
assumptions growth rates of the microorganisms in the temperature range of (0 to 30) °C were predicted using the data obtained from ComBase (http://modelling.combase.cc/). Maximum storage time, time for which the microbial count did not exceed the allowable limit, was evaluated for each microorganism at different temperatures. The microorganism that exceeded the spoilage limit sooner, with the faster growth rate, was determined and considered as the representative microorganism for further studies.

3.6.2 Microbial kinetics

Microbial kinetics was evaluated for the representative microorganism in beefsteak. ComBase database was used for the kinetics evaluation. With the previously mentioned initial microbial load and spoilage limit assumptions, microbial growth level of the specific organism in terms of log increase was predicted at five different temperatures ranging from 0 °C to 30 °C. For safety and convenience, the lag factor associated with the microbial growth was disregarded, assuming instantaneous growth when the product temperature exceeds final thawing point, so that linear relation could be obtained. With these data, the growth level at each temperature was evaluated using equation (3.16). Arrhenius equation (3.17) was fitted to the Arrhenius plot of 1/T vs. 1/k to evaluate the pre-exponential factor A and activation energy $E_a$.

\[
\log \left( \frac{N}{N_0} \right) = kt \tag{3.16}
\]

\[
k = A e^{-\frac{E_a}{RT}} \tag{3.17}
\]
where $N_0$ and $N$ are initial and final microbial load in CFU/g, respectively, $k$ is the microbial growth constant, time $t$ in seconds, $E_a$ is activation energy expressed in J/mol, $A$ the pre-exponential factor in s$^{-1}$, $R$ has a constant value of 8.314 J/(mol · K) and temperature $T$ is in K.

### 3.6.3 Microbial quality prediction

Microbial growth kinetics developed using ComBase was incorporated into COMSOL® Multiphysics program to predict the microbial growth. The extent of microbial growth in the food system was evaluated at the end of any given time, with respect to the temperature distribution at a particular location. Microbial growth prediction was done at the most sensitive region of beefsteak, the region where temperature will always be higher than other regions, hence will get microbially spoiled first. Generally corners are the regions that thaws first. By analyzing the temperature distribution of all the corners in five beefsteaks, the corner where the temperature was higher at any given time was determined. This region was termed as sensitive region. A small volume at the surface of the sensitive corner of the beefsteak, was selected for the microbial study. Shape of the small region was assumed to be a cube of each side measuring 8 mm. Since minimum size of a tetrahedral mesh element was around 4mm, length of 8 mm can account at least two nodal temperatures in all three directions. Equation (3.18) was used to calculate the volumetric microbial log increase on the cubic volume of the beefsteak in the sensitive region.
\[
\log\left(\frac{N}{N_0}\right) = \frac{1}{V} \iint k \, dv \, dt = \frac{1}{V} \iint \left(Ae^{(-E_a/RT)}\right)dv \, dt
\]

(3.18)

where \(V\) is the total volume of the sensitive region used for analysis. With the known assumptions, if microbial level exceeds the maximum allowable limit, food was considered microbially unsafe.

### 3.7 Thawing time

In this study, thawing time is the time taken by the frozen food at \(-18\) °C to start thawing, i.e., to reach final thawing point, which is approximately \(0\) °C. Microbial growth dominates once the temperature is above this final thawing point. Dependency of thawing time on external temperature was evaluated by exposing the frozen menu box to different constant external temperatures (20, 25, 30, 35, and 40) °C and predicting the thawing time of each product at the sensitive region. As mentioned in the previous section, temperature sensitive corner regions for each food item was identified. Since the minimum distance between two nodes is 4 mm, temperature data of a point distancing 4 mm from all the three sides at that sensitive corner was considered as the sensitive region temperature.

### 3.8 Freezing time

Freezing time is the time taken by the thawed food to freeze back to initial freezing point from its actual temperature. Though the product temperature is reducing during freezing time, since the temperature is above initial freezing point it will
significantly contribute to the microbial growth. Freezing time calculation was done at the sensitive region of beefsteak. Thawed beef steak in a menu box at different temperatures, were exposed to constant -18 °C to develop a correlation between the product temperature and freezing time.

3.9 Case studies

Two possible temperature fluctuation/abuse scenarios during cold chain transportation are (i) high ambient temperature exposure for prolonged time while shifting between two cold systems and (ii) freezer breakdown. The validated numerical simulation algorithm for heat transfer was combined with the microbial growth kinetics to simulate the end microbial quality of the frozen army menu box under these scenarios. External heat transfer coefficient evaluated based on experimental validation was applied on the outer walls of the menu box during freezing and thawing. Short description on each case and the conditions used for numerical simulation are discussed in the following section.

3.9.1 Case 1: Menu box exposed to constant high external temperature

The goal of case 1 was to predict the maximum allowable time for the exposure of menu box to constant high ambient temperatures before the food became microbially unsafe. Microbial growth during temperatures above the final thawing point while thawing and temperatures above initial freezing point while freezing back were considered. With the numerical simulation, microbial count was predicted at the sensitive region of beefsteak in the menu box. The menu box was initially frozen at -18 °C and
was exposed to different constant ambient temperatures: (20, 25, 30, 35, and 40) °C. Different exposure time was tried to evaluate the maximum allowable time at each temperature, before the box was put back in freezer of -18 °C. In this case the products were suddenly exposed to ambient and freezing temperatures. The spoilage limit was calculated with the microbial assumptions made in section 3.6.1.

3.9.2 Case 2: Freezer breakdown

The goal of case 2 was to predict the maximum allowable freezer breakdown time before the beefsteak in the menu box became microbially unsafe. In the freezer breakdown case, unlike the previous case, the external temperature changed gradually. The products were initially at -18 °C inside the freezer. When the freezer broke down, the temperature inside the freezer rose slowly to surrounding ambient temperature and once the freezer was restarted, the temperature gradually dropped back to -18 °C. Similar to the previous case, different freezer breakdown time was tried to evaluate the maximum allowable freezer breakdown time. This evaluation was based on microbial safety prediction. The rate at which the temperature rose and fell was obtained from an on-field freezer breakdown case data, from the UGR-A transportation system. Monitored temperature variations in a loaded container is shown in Table 3.3.

In both these cases, thawing and freezing conditions had to be used in the same numerical simulation program. From DSC, the thermo-physical properties of the food items were obtained for both freezing and thawing processes, individually. The properties of the food were defined based on the process, either thawing or freezing, it underwent at
that particular time 't'. This condition was imposed in COMSOL® Multiphysics by *if* function. In each node *if* \(dT/dt\) is greater than 0, i.e., change of temperature with respect to time was positive, then the food underwent thawing process and possessed thawing properties else the food possessed freezing properties.

**Table 3.3: Environmental real time temperature fluctuation data**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-5</td>
</tr>
<tr>
<td>96</td>
<td>+7</td>
</tr>
<tr>
<td>176</td>
<td>+7</td>
</tr>
<tr>
<td>188</td>
<td>-17</td>
</tr>
<tr>
<td>244</td>
<td>-17</td>
</tr>
</tbody>
</table>
4. RESULTS AND DISCUSSIONS

Initially, enthalpy thermograms of all food items were obtained using DSC to evaluate their thermo-physical properties, which were used in the numerical simulation. Later, the numerical simulation program was validated by comparing the numerically predicted temperature history with the experimental data. Subsequently, microbial kinetics were evaluated and incorporated in the numerical simulation to predict food safety, from which the extent of temperature abuse that a menu box can undergo before becoming microbially unsafe under possible temperature fluctuation scenarios, was estimated.

4.1 DSC thermogram

DSC thermograms were generated for five different food items and two different model food systems. Figures 4.1a and 4.1b show the average heat flow curves of five different food items during freezing and thawing respectively. The values in parenthesis represent the total moisture content of each food product. The heat flow was expressed in W/g. Latent heat released during freezing was represented by the area of the peak in positive direction (Fig. 4.1a), and during thawing, the curves were in negative direction due to absorption of latent heat (Fig. 4.1b). The area under the peak represents the amount of energy exchanged during phase change. It was noticed that the height of the peak is proportional to the total moisture content. The higher the water content, the higher the energy flow during phase change.
Figure 4.1: DSC thermograms obtained during (a) freezing and (b) thawing for five different food items. Temperature changing rate was set at 10 K/min.

Though orange juice and beefsteak had approximately same proportion of water, heat flow peak of beefsteak was taller. This suggested that beefsteak had less bound
water, so most of its water fraction had undergone phase change unlike orange juice. It has been shown that, with more soluble solids the height of the peak reduces (Thanatuksorn et al., 2008). This might be the reason for comparatively lower peak of orange juice which contains high amount of soluble sugars. In case of danishes, no noticeable peak was observed. This may be due to its comparatively low moisture content, and most of the moisture present must be in the form of bound water that did not undergo any phase change.

Figures 4.2a and 4.2b represent freezing and thawing heat flow curves of two different model food systems. As expected 25 % gel, with 75 % moisture content (w.b.) has taller peak than 30 % gel.

![DSC thermograms obtained during freezing of two different types of gels. Temperature changing rate was set at 10 K/min.](image)

Figure 4.2a: DSC thermograms obtained during freezing of two different types of gels. Temperature changing rate was set at 10 K/min.
Figure 4.2b: DSC thermograms obtained during thawing of two different types of gels. Temperature changing rate was set at 10 K/min.

Freezing thermograms show that, freezing process takes place in the range of -12 °C to -24 °C. As mentioned earlier, this anomaly can be attributed to super cooling and higher temperature changing rate (Le Reverend et al., 2008). To obtain actual initial freezing point, freezing experiments were done for five food items and two gel systems. Results of beefsteak are discussed in this section. Figure 4.3 represents the freezing curve of beefsteak, which is a typical temperature history during freezing. The graph can be divided into three regions: temperature drop in thawed liquid phase, plateau phase change region and temperature drop in solid frozen phase. The temperature drop in liquid and solid phase was due to constant sensible heat removal. In the phase change region, freezing of beefsteak started at -1.9 °C and was complete at -3.6 °C. The temperature gradient in the phase change region was small due to large amount of energy released to
freeze beefsteak. This excess energy is latent heat of freezing (Foong et al., 2010). From this graph the initial freezing point of beefsteak was found to be -1.9 °C. The DSC freezing curve (Fig. 4.1a) of beefsteak was shifted such that the peak is at -1.9 °C. Similarly freezing experiments were done for all the food items and the DSC curves were shifted accordingly. Later, these shifts were experimentally validated.

![Figure 4.3: Freezing experiment of beefsteak. Initial freezing point shown as a black dot.](image)

4.2 DSC analysis

The thermo physical properties explained in the materials and methods section 3.2, were evaluated from the DSC thermograms. Apparent Specific Heat (ASH) curves were plotted using equation (3.1), with the temperature changing rate of 10 K/min. Figure 4.4a displays the results for the corrected ASH curves during freezing, shifted according to the experimental initial freezing point and Fig. 4.4b shows the results for thawing curves. ASH curves during freezing had a narrow peak width when compared to long
tailing peak while thawing. This indicated that during thawing, phase change occurred over a wider range of temperature and more gradually when compared to freezing. Similar ASH curves were derived for the two model food systems.

Figure 4.4: Apparent specific heat curve for (a) freezing (b) thawing processes of five different food items
The latent heat of phase change, i.e., the area under the curve was approximately equal for all food items in thawing and freezing curves. This confirms that same amount of moisture had undergone phase change during freezing and thawing. From the latent heat of phase change value, bound water fraction was calculated for each food item using equation (3.2). Experimentally evaluated initial freezing points and final thawing points that were obtained from DSC thawing curves, the latent heat of phase change and the bound water fraction of the four food items and the two model food systems, are shown in Table 4.1. As mentioned earlier danishes did not undergo phase change.

**Table 4.1: Latent heat of phase change and bound water fraction of food items**

<table>
<thead>
<tr>
<th></th>
<th>Initial freezing point (°C)</th>
<th>Final thawing point (°C)</th>
<th>Latent heat of phase change (J/(g · K))</th>
<th>Bound water fraction (x_b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beefsteak</td>
<td>-1.9</td>
<td>-0.5</td>
<td>153.18</td>
<td>0.17</td>
</tr>
<tr>
<td>Orange juice</td>
<td>-4.5</td>
<td>-2.5</td>
<td>123.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Peppers and onions</td>
<td>-3.2</td>
<td>-1.9</td>
<td>229.77</td>
<td>0.16</td>
</tr>
<tr>
<td>French toast</td>
<td>-1.2</td>
<td>-1</td>
<td>86.58</td>
<td>0.08</td>
</tr>
<tr>
<td>25 % gel</td>
<td>-0.7</td>
<td></td>
<td>240.43</td>
<td>0.03</td>
</tr>
<tr>
<td>30 % gel</td>
<td>-0.8</td>
<td></td>
<td>204.84</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Based on the colligative properties, due to high amount of soluble sugar content, orange juice had lowest initial freezing point and final thawing point (Thanatuksorn et al., 2008). When comparing the high moisture systems: peppers & onions (85 % moisture) and 25 % gel (75 % moisture), the latent heat of phase change was higher in 25 % gel. This may be due to the homogenous nature of gelatin system and less bound water fraction, whereas in the heterogeneous peppers and onions system, the possibility of water entrapped in the food matrix is higher, i.e., higher bound water fraction when compared to gel.

Since the shape of the peak is different for freezing and thawing, the rate of latent heat release/absorption is different. So, ice fraction and unfrozen water fraction as a nonlinear function of temperature were calculated separately for freezing and thawing cycles. Figures 4.5a and 4.5b show the unfrozen water fraction as a function of temperature for the four food items during freezing and thawing. In both the graphs, at -30 °C, the water fraction was at constant bound water fraction value, and at the respective initial freezing/final thawing point water fraction were at its maximum value, i.e., total moisture content (w.b.) which corresponds to the liquid form. The total moisture content of each food item is mentioned in parenthesis. Depending on the process, freezing or thawing, the corresponding curve was chosen for numerical simulation. Similarly for the two types of model food systems, the unfrozen water fraction and the ice fraction curves were obtained as a function of temperature.
Figure 4.5: Unfrozen water fraction as a function of temperature during (a) freezing and (b) thawing

The density and the thermal conductivity of each food item in the three regions, frozen, mushy and thawed, as a function of temperature were evaluated from the combination of their individual components. Data from Table 3.1 and Table 3.2 were used
for this evaluation. Values from unfrozen water fraction and ice fraction curves were included in these calculations. The correlations for freezing and thawing processes were developed separately and used accordingly in numerical simulation.

4.3 Experimental validation

The purpose of experimental validation was to validate the numerical approach, the governing equations, the boundary conditions and the simplifications imposed. Results of the experimental verification which were carried out using model food systems and real food systems are explained in this section.

4.3.1 Thawing experiment with model food system

Four different thawing experiments were carried out with the frozen model food systems: 25% gel thawing in an ambient environment, 25% gel thawed inside an insulated box, 30% gel thawing in an ambient environment, 30% gel thawed inside an insulated box. Temperatures at four known locations, along the axis of the gel were monitored and studied. The experimental temperature variations were compared with numerically predicted temperature data using three different curves to obtain ASH. Figure 4.6 shows the comparison results at location 2 (Fig. 3.9c) in the model food system for the four thawing experiments. As discussed earlier, the temperature data has three regions: initial temperature rise zone in the frozen gel, plateau phase change region and finally temperature rise zone in thawed gel. The change of slope around the 0 °C in experimental data (black dotted line) represents the completion of phase change, i.e., final thawing point.
Figure 4.6: Experimental validation of model food system at location 2
   a) 25 \% gel left in ambient   b) 25 \% gel inside a insulated box
Figure 4.6: Experimental validation of model food system at location 2

(c) 30% gel left in ambient       (d) 30% gel inside a insulated box

It could be inferred from the experimental validation of model food system that the cubic polynomial fit and the heaviside function, fitted better with the experimental data than the derivative method curve. This could be attributed to the fact that the
derivative method recalculates the curve using specific heat and latent heat of phase change from components, whereas the cubic polynomial method and the heaviside method used actual experimental curve from DSC ASH data. 100% matching was not expected between the experimental curve and numerical prediction curves, due to the assumptions made and high temperature changing rate during DSC study (Le Reverend et al., 2008).

4.3.2 Thawing experiment with real food system

Thawing experiments were carried out on the frozen menu box with all the food items arranged in the fixed orientation. The frozen menu box was allowed to thaw at ambient temperature conditions and product temperatures were monitored at four known locations in beefsteak, orange juice, peppers and onions and French toast as shown earlier in Fig. 3.8. The experimental temperature profile was compared with those from the numerical simulation using three different ASH curve methods. Figure 4.7 shows the comparison of temperature variation with time for the four food items at known locations. It can be seen that the cubic polynomial method fits the experimental data better than the other two functions. As found earlier, the derivative method showed the greatest deviation from experimental data. The better accuracy of the cubic polynomial method when compared to heaviside function is because cubic polynomial method precisely regenerated the DSC ASH curve by an equation, whereas piecewise heaviside function generated a step function and failed to incorporate the trend of the peak during phase change. More deviations between the experimental data and numerical predictions for the
beefsteak data (Fig. 4.7a) were due to the challenges in real time temperature measurement. Position of the thermocouple was not stationary throughout the process as it got disturbed during thawing of food.

Figure 4.7: Experimental validation of frozen real food system at 4 known locations (a) Beefsteak (b) Orange juice
In the case of orange juice (Fig. 4.7b), the fluctuations in real time data (black dotted line) were due to the natural convection of thawed orange juice, which was not accounted for in the simulation. Deviations in French toast data (Fig. 4.7d) may be due to
the assumption of simplifying the geometry, in which twenty four individual French toasts were considered as one rectangular slab. It was expected that the deviation in the predicted curves of real food system would be greater than that for the model food system. This can be attributed to the fact that model food system is homogenous in nature and gelatin is freeze thaw stable whereas real food system is complex and heterogeneous in nature. The observed predictive capacity of the numerical simulation for real food system was considered as reasonable.

More importantly, the experimental validation was used to optimize the heat transfer coefficient of ambient environment over the outer surface of the menu box. The best results were obtained when the heat transfer coefficient value of 20 W/(m$^2$ · K) on the exposed sides of the box and 5 W/(m$^2$ · K) on the base, were used.

**4.3.3 Freezing experiment**

The model for freezing process was developed using shifted ASH curves. This model was validated by comparing the time temperature data obtained from the freezing experiment with numerically predicted results. The experimental verification was carried for freezing behavior of beefsteak. The experimental temperature data of beefsteak at a known location, while freezing, were compared with the numerically simulated results. The simulation was carried out using cubic polynomial fit of ASH curve, since it was found to be the best, as explained earlier. Heat transfer coefficient values mentioned in the previous section were used in the simulation. The comparison result at a known location is shown in the Fig. 4.8. The good agreement between predicted and
experimental temperature variations confirmed that modifications made in heat flow curve were reasonable. It also verified the numerical solutions obtained using cubic polynomial fitted ASH method.

![Experimental temperature variations](image)

**Figure 4.8: Experimental validation of freezing process of beefsteak at a known location**

Once, the numerical simulation program developed for the frozen army rations using DSC and component data was validated, further research on the prediction of microbial quality of food products during temperature abuse conditions was carried out.

### 4.4 Microbial study

Five microorganisms considered for this study were *Escherichia coli* O157 H7, *Salmonellae, Listeria monocytogenes, Clostridium botulinum* and *Pseudomonas spp.*. Individual behavior of these organisms at different temperatures on the conditions similar to beefsteak was evaluated using ComBase. Maximum storage time was calculated based
on the allowable limit of the microbial count as explained in section 3.6.1. Figure 4.9 shows the dependency of maximum storage time over the temperature with respect to different microorganisms.

![Figure 4.9](image)

**Figure 4.9: Maximum storage time as a function of product temperature. -Influence of different microorganisms over the substrate resembling beefsteak**

At higher temperature, microbial growth is faster, so the storage time is less, before the food became microbially unsafe. It was found that *Pseudomonas spp.* had higher growth rate in beefsteak, hence lowest storage time at a given temperature. If the product is safe based on the *Pseudomonas spp.* growth, then the product should be safe from all the other organisms. A detailed study in evaluating the kinetics of *Pseudomonas spp.* was carried out.

**4.4.1 Microbial growth kinetics**

*Pseudomonas spp.* is a spoilage microorganism, so as mentioned earlier, initial
microbial load of $10^5$ (CFU/g) and allowable limit of 2.5 log increase were assumed to evaluate its growth kinetics in beefsteak. Growth rates of this microorganism under different temperatures were analyzed in detail. Growth kinetics were evaluated by plotting microbial log increase (CFU/g) at different temperatures against time as shown in Fig. 4.10.

**Figure 4.10: Pseudomonas spp. count increase at different temperatures - modeled using ComBase**

Microbial growth started immediately after time $t=0$, and the trend was linear, because of the safer assumption of no lag time. As expected, the growth rate increased with increase in temperature. Growth rate of *Pseudomonas spp.* at each temperature was evaluated by fitting a linear equation to each curve. Dependence of growth rate on temperature was modeled using Arrhenius equation (3.17). The Arrhenius plot of $1/T$ vs $\ln(k)$ is shown in Fig. 4.10. By fitting the Arrhenius equation to the curve in the graph,
the values of Arrhenius constant $A = 1.403 \times 10^9 \text{ s}^{-1}$ and activation energy $E_a = 7.423 \times 10^4 \text{ J/mol}$ for *Pseudomonas spp.*, were obtained. This growth kinetics was incorporated with the temperature data obtained from the numerical simulation for food safety prediction.

![Arrhenius plot](image)

**Figure 4.11: Arrhenius plot of *Pseudomonas spp.* ($R^2 = 0.9981$)**

4.5 Temperature sensitive region

The most temperature sensitive region of beefsteak was selected to carry out microbial study. This region was expected to thaw first and therefore microbially spoil first. Figure 4.12a highlights the most sensitive region of beefsteak. A small cubic volume at the surface of the sensitive corner of the beefsteak, as shown in Fig. 4.12b, was selected for the microbial study. With the known *Pseudomonas spp.* kinetics, volumetric log increase of that specific organism was evaluated in the sensitive region. Evaluation was done with respect to the average time-temperature data at that region using equation (3.18).
Figure 4.12: (a) 3D geometry of menu box - Temperature sensitive region is highlighted by a red dot (b) 3D geometry of Beefsteak - analyzed small volume in the sensitive region is highlighted

4.6 Thawing time

Thawing is a process where the frozen ice in food undergoes phase change and becomes liquid water. In this study, the term thawing time refers to the time taken by frozen food at -18 °C to start thawing, i.e., time at which the temperature reaches final thawing point. Depending on the material properties, the orientation of the food item, and the external temperature, the thawing process and the thawing time vary for each food item in the menu box. The thawing process predicted from the numerical simulation is explained below. The frozen menu box, at -18 °C, as described in Fig. 4.13 was exposed to a constant external temperature of 30 °C. The temperature distribution within the menu box at different time intervals is shown in Fig. 4.14.
Figure 4.13: 3D geometry of menu box with description.

Figure 4.14: Temperature distribution within frozen menu box during thawing when exposed to 30 °C at different time intervals.
Figure 4.14: Temperature distribution within frozen menu box during thawing when exposed to 30 °C at different time intervals.
In Fig. 4.14, the colors represent temperature levels, blue being the coldest and red being the warmest. When a frozen menu box at -18 °C is exposed to external constant temperature of 30 °C, the evolution of temperature distribution can be followed from these figures. At time t=0, the box is completely frozen, so represented in blue color. Once the box was exposed to 30 °C, the temperature of air in the headspace started rising immediately as we can see in the Fig. 4.14 at t=10 min, but all the food items were still in frozen state. The air in the headspace of beefsteaks box was colder when compared to air in the other parts of box, this was due to the heat transfer from the coldest beefsteaks to the headspace air. At this time danishes at the back of the box were at maximum temperature because danishes were closer to the external surface and were immediately exposed to high surrounding temperature. Also, danishes had very low moisture and did not experience any phase change, so most of the heat transfer in danishes was due to its dry food components. Hence the danishes reached 0 °C first. At t=30 min, the corners of orange juice and peppers & onions containers were approaching 0 °C range, which indicates the beginning of thawing. After 1 hour more than 50 % of orange juice and peppers & onions were thawed, and corners of French toast were approaching 0 °C. At this stage, the temperature of danishes were completely above 0 °C and beefsteaks were still in frozen state. After 5 hours orange juice, peppers & onions were completely thawed. Corners of beefsteaks had started to thaw, indicating their onset of thawing between 1 h to 5 h and 50 % of French toast was thawed. In the next three figures, at t=10 h, 20 h, and 30 h, the movement of thawing front from corner of products towards
the center can be clearly seen; the region in blue denotes the temperature of <5 °C. At 20 h, all the food items except beefsteak 3 were thawed completely. At 30h all the food items had thawed completely. Except for beefsteak, all other food items were approaching surrounding temperature of 30 °C.

Thawing time was calculated at the most sensitive part of each food product. Thawing times for each product when menu box was exposed to different external temperature are plotted in Fig. 4.15. For danishes, time at which it reached 0 °C was mentioned as thawing time.

![Graph showing thawing time of various food items](image)

**Figure 4.15: Thawing time of all the food items when exposed to different constant external temperatures.**

As the external temperature was increased, the thawing times of products decreased. Danishes, orange juice, peppers and onions had very less dependence on the external temperature. This may be because, these food items were nearer to the external surface of the box and were immediately exposed to high temperatures. So irrespective of
the level of the external temperature, they thawed in the shortest time. The thawing times of beefsteak and French toast showed greater dependence on the external temperature, and beefsteak being the slowest one to thaw. Thus the thawing time of each food product depended on the type of food, orientation/location inside the box and the external temperature.

4.7 Freezing time

Freezing process was simulated using very low ambient temperature of -18 °C. Initial freezing point was used to evaluate the freezing time. The temperature of the product will depend on the exposure time to high external temperature. Higher the exposure time, the greater the product temperature and the longer the freezing time. Dependency of freezing time over the product temperature was studied at the sensitive region of beefsteak and the correlation is showed in Fig. 4.16.

Figure 4.16: Relation between freezing time and product temperature at the sensitive region of beefsteak
Temperature sensitive region is the region where the temperature is always higher when compared to other regions; this region was evaluated based on thawing process. During freezing this region freezes first, since it is the most sensitive region to external temperature variations. But for the purpose of this project, while analyzing targeted temperature abuse scenarios (explained in the next section), the temperature sensitive region experienced higher microbial growth when compared to the colder center regions and relatively equal microbial growth when compared to the warmer nearby regions. Hence, for convenience this temperature sensitive region was analyzed to evaluate freezing time and product temperature correlation.

4.8 Case studies

The heat transfer model was successfully developed and validated with the experiments. Microbial kinetics of the most dominant microorganism, *Pseudomonas spp.* was evaluated. A protocol to couple the kinetics with the predicted temperature was developed. The behavior of food products during thawing and freezing was understood well. From this knowledge a relationship between the microbial quality and possible temperature abuse scenarios was to be developed so that the end quality of the food product could be judged.

4.8.1 Case 1 - Menu box exposed to constant high external temperatures

Exposure of a frozen menu box to various constant external temperatures (20, 25, 30, 35, and 40 °C) was simulated. Microbial growth level of *Pseudomonas spp.* in the sensitive region of beefsteak, was predicted for these cases. As mentioned in materials
and methods section 3.9.1, the exposure time of menu box to high temperature was varied on to evaluate the spoilage time. When the products were put back in the freezer, the microbial growth during freezing time was also considered. Figure 4.17 explains the thermal behavior of beefsteak, when the menu box was exposed to 25 °C for more than a day and later moved back to freezer at -18 °C.

![Time-temperature profile of most sensitive point in beefsteak](image)

**Figure 4.17: Time-temperature profile of most sensitive point in beefsteak**

In Fig. 4.17, the black dotted line represents the external temperature, which was held at 25 °C for 25 h and abruptly changed to -18 °C. The red curve shows temperature variation of the sensitive region of beefsteak. Temperature of the frozen beefsteak, which was initially at -18 °C rose till the boxes were moved back to freezer. The dotted red line represents projected temperature profile for increased exposure time. The time required
for 2.5 log increase in *Pseudomonas spp.* (allowable limit) population at the sensitive region of beefsteak during thawing was calculated and this time was termed as spoilage time, $T_s$. This is the time before which the product should be frozen back below its initial freezing point (approximately 0 °C). Freezing time was denoted as $T_f$. The extent of microbial growth during freezing back time was approximately equal to microbial growth during excess exposure time (dotted red line). Therefore, an assumption was made that contribution to microbial growth by freezing time and excess exposure time was equal, thus the maximum allowable exposure time ($T_m$) was approximately given by $T_m = T_s - T_f$.

Figure 4.18 shows microbial growth curves of the most sensitive region in beefsteak when exposed to different external temperatures.

**Figure 4.18: Microbial log (CFU/g) increase at sensitive region of beefsteak during exposure of menu box to different external temperature**
Spoilage time ($T_s$), time at which the microbial log (CFU/g) increase reached 2.5 at each temperature, was obtained from this graph.

In each external temperature case, the freeze back time ($T_f$) for the respective maximum temperature of the product was calculated from the correlation in Fig. 4.16. Spoilage time ($T_s$) and freezing time ($T_f$) are tabulated in Table 4.2. Maximum allowable time ($T_m$) was calculated by subtracting freezing time from spoilage time.

### Table 4.2: Maximum allowable time at each temperature (based on beefsteak)

<table>
<thead>
<tr>
<th>External temperature ($^\circ$C)</th>
<th>Spoilage time (h) $T_s$</th>
<th>Freezing time (h) $T_f$</th>
<th>Maximum allowable time (h), $T_m=T_s-T_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>29.8</td>
<td>1.5</td>
<td>~28</td>
</tr>
<tr>
<td>25</td>
<td>22.7</td>
<td>2.4</td>
<td>~20</td>
</tr>
<tr>
<td>30</td>
<td>18.2</td>
<td>2.9</td>
<td>~15</td>
</tr>
<tr>
<td>35</td>
<td>15.5</td>
<td>3.5</td>
<td>~11</td>
</tr>
<tr>
<td>40</td>
<td>13.2</td>
<td>4.1</td>
<td>~9</td>
</tr>
</tbody>
</table>

Maximum allowable time was plotted against external temperature as shown in Fig. 4.19. At a given temperature, if the exposure time is above the curve, the food is microbially spoiled and if the temperature abuse time falls below the curve, the food is considered as microbially safe.
Case 2 - Freezer breakdown:

In the freezer breakdown case, the external temperature was allowed to change gradually. The rate of change of external temperature was obtained from an on-field real case, as explained in Table 3.3. The rates of change of temperature evaluated by extrapolating these data were as follows. For temperature rise from -18 °C to 0 °C the temperature changed at a rate of 0.125 °C/h, and from 0 °C to any positive value the rate of change of temperature was 0.216 °C/h. While cooling back when freezer started working, irrespective of temperature range, the rate was assumed to be -2 °C/h. Figure 4.20 explains the thermal behavior of the beefsteak, when the frozen menu box was inside a broken freezer for 10 days and was fixed at the end of 10 days.

Figure 4.19: The relation between external temperature and maximum allowed exposure time
Figure 4.20: Time-temperature profile for freezer breakdown scenario when the freezer was broken for 10 days.

In Fig. 4.20, the black dotted line represents the external temperature experienced by the product inside the freezer during breakdown. The red line represents the temperature variation of the sensitive region of beefsteak for freezer breakdown scenario. In the broken freezer, the temperature inside the freezer rose slowly because of which the product temperature was also increased gradually. When compared to the previous case, the product took relatively longer time to reach 0 °C. Once the freezer was restarted/repaired, the temperature inside freezer quickly dropped to -18 °C. Because of the relatively sharp decline in freezer temperature, the product froze back in comparatively shorter time. The microbial quality evaluation was carried out for this time temperature data as described in previous case. The time required for 2.5 log increase of
*Pseudomonas spp.* (allowable limit) at the sensitive region of beefsteak had to be obtained. Freezer break down time was varied to evaluate spoilage time ($T_s$), freezing time ($T_f$) and maximum allowable freezer breakdown time ($T_m$). Since the slopes of external temperature change were fixed, there could be only one possible solution for this problem.

Figure 4.21 shows the microbial growth at the most sensitive region of beefsteak during freezer breakdown scenario. Spoilage time ($T_s$) at which the microbial count reached 2.5 log increase was about 200 h. The freeze back time ($T_f$) for the product at that maximum temperature was around 14 h, which was obtained by extrapolating the curve in Fig. 4.16. Thus the maximum allowable breakdown time ($T_m$), was obtained by subtracting the freezing time from the spoilage time, which was around 186 h.

![Figure 4.21: Microbial growth profile during freezer breakdown scenario](image)
5. CONCLUSIONS

5.1 Conclusions

Numerical simulation of freeze-thaw process was carried out to analyze the effects of temperature abuse conditions on the microbial quality of frozen military rations during storage and transportation. The simulations were carried out using commercially available Finite Element Method (FEM) based software COMSOL® Multiphysics version 4.2b. The numerical simulation program developed was validated using experimental data. The experimental results showed a reasonable agreement with the numerical predictions. The model successfully incorporated phase change behavior, complex 3D geometry of the menu box and that of the food products, their orientation, natural convective airflow in the headspace of the box, and packaging material properties. Differential Scanning Calorimetry (DSC) was used to extract the thermo-physical properties of the food items to develop the numerical simulation program. In spite of few drawbacks, DSC helped in obtaining reasonably accurate material properties of the food items. Among the three methods used to incorporate Apparent Specific Heat (ASH) curve in the model, cubic polynomial exponential fit of the DSC data was found to be the most suitable.

Microbial quality of the food product was evaluated, since chemical degradation was comparatively considered to be negligible. Beefsteak was assumed to be the most susceptible food item for microbial spoilage. So, microbial study was done in beefsteak. Growth rates of different possible microorganisms were evaluated using ComBase
database, among which spoilage microorganism *Pseudomonas spp.* was found to be the dominant microorganism in beefsteak. The kinetic model of *Pseudomonas spp.* was successfully combined with the heat transfer numerical simulation to predict the microbial quality. Microbial quality prediction was done for two possible temperature abuse scenarios: (i) constant high external temperature exposure and (ii) freezer breakdown. For the exposure of frozen menu box to constant high external temperatures of (20, 25, 30, 35, and 40) °C, the menu box can be allowed to stay at those temperature for a maximum time of (29, 20, 15, 12, and 9) h, respectively, to ensure microbially safe food. In case of freezer breakdown, the menu box can be allowed to stay in the broken freezer for a maximum time of 186 hours to ensure food safety.

A generic numerical simulation technique was developed which can be used for analysis of any frozen food system. This technology can assist the food industry and related risk assessors in evaluating the temperature oriented risk profile of perishable food products. The data can enable the industry to maximize product quality, while minimizing the food safety risk caused by potential temperature abuse that occurs after a product leaves the food manufacturing facility.
6. FUTURE WORK

In future research, the following aspects can be considered.

1. From the results obtained, we propose to design a Time-Temperature Integrator (TTI), which based on kinetics of microbial growth and heat transfer prediction, would indicate whether the product is suitable for consumption or not.

2. Similar to the microbial quality prediction, analysis of chemical quality of food items can also be done with the respective chemical kinetics.

3. The numerical simulation technique has to be extended to study the effect of temperature fluctuation scenarios over a pallet of frozen boxes during storage and transportation. More computational power will be needed to predict the results.

4. Due to possible heat flow between the food items in the box, effect of change in the orientation of food items inside the box over the microbial quality of food have to be analyzed.
7. REFERENCES


APPENDIX

The ingredient list of the five targeted food items:

**USDA - select Beef sirloin tri tip steak**

Ingredients: Contains up to 12% of a solution of water, salt. Seasoning (potato maltodextrin, natural flavor, gum arabic), sodium phosphates, beef stock.

**Citrus Belle - Frozen concentrated Orange juice**

Ingredients: Orange juice concentrate, water.

**RoastWorks - Flame roasted Peppers and Onions**

Ingredients: Onions, bell papers, soybean and/or sunflower oil, seasoning (cornstarch, salt, dehydrated garlic and onion, sugar, hydrolyzed corn gluten, spices, refinery syrup [molasses, caramel color], yeast, modified cellulose, natural flavors [{contains soybean and wheat}, maltodextrin, modified food starch, corn syrup solids, yeast extract, salt, dextrose, tricalcium phosphate, citric acid], citric acid, natural flavors, yeast extract, caramle color, butter, oleoresin of paprika)

**Tornados - French toast flavored taquito with maple flavored sausage wrapped in a battered flour tortilla**

Ingredients: Sausage link (pork, water, salt, maple sugar, spices, dextrose, maple flavor (caramelized sugar, syrup, flavorings, maple syrup, brown sugar), sugar), water, wheat flour (enriched with niacin, reduced iron, thiamin mononitrate, riboflavin, folic acid), vegetable oil (soybean, sunflower, canola and/or corn oil), batter milk (wheat flour, yellow corn flour, dextrose, cornstarch, sugar, tapioca dextrin, eggs, nonfat milk,
leavening (sodium aluminium phosphate, sodium bicarbonate), salt, modified food starch, natural and artificial flavors, spice, sucrrose, guar gum, maple syrup, brown sugar molasses), maltodextrin, tortilla flour blend (salt, rice flour, guar gum, wheat, sodium bicarbonate, corn starch, wheat starch, food starch, dough conditioners (sodium metabisulfite, sodium stearoyl lactylate, L-cysteine, mono and diglycerides, dicalcium phophate), microcrystalline cellulose, silicon dioxide (to prevent caking)), dextrose.

**Pepperidge farm - Mini assorted Danish**

Ingredients: Unbromated unbleached enriched wheat flour [flour, malted barley flour, niacin, reduced iron, thiamin mononitrate (vitamin B1), riboflavin (vitamin B2), folic acid], water, sugar, margarine [partially hydrogenated soyabean and cotton seed oils, water, salt, vegetable mono and diglycerides, nonfat milk, soy lecithin, artificial butter flavor, beta carotene (color), vitamin A, palmitate added], corn syrup, bleached enriched flour [flour, niacin, reduced iron, thiamin mononitrate (vitamin B1), riboflavin (vitamin B2), folic acid], partially hydrogenated vegetable shortening (Soybean and cotton seed oils), whole eggs, neufchatel cheese (pasteurised milk and cream, cheese culture, salt, carob bean gum). Contains 2 percent or less of: yeast, apples, brown sugar, dextrose, red raspberries, invert sugar, cinnamon, salt, degerminated yellow corn flour, modified food starch, sodium stearoyl lactylate, calcium sulfate, leavening (sodium acid pyrophosphate, baking soda), nonfat milk, lemon juice concentrate, natural and artificial flavors, whey, citric acid, raspberry juice concentrate, vegetable mono and diglycerides, agar, soybean oil, apple juice concentrate, lemon peel, lemon oil, titanium dioxide (color), cornstarch,
extractives of turmeric (color), paprika (color), locust bean gum, polysorbate 60, calcium carbonate, plum juice concentrate, yellow 5, yellow 5 lake, yellow 6 lake, yellow 6, glycerol monooleate, malted barley flour, sodium citrate, tragacanth gum, carageenan, propyl gallate, alpha tocopherol, potassium sorbate and sodium benzoate (preservatives), apo carotenal and beta carotene (color).