DEVELOPMENT OF MATHEMATICAL MODELS FOR THE PREDICTION OF SHELF LIFE OF CHEESE - PURI MIX

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRYING

(DAIRY ENGINEERING)

By

THANUJA D.

B. Tech. (Dairy Science and Technology)

DAIRY ENGINEERING SECTION

NATIONAL DAIRY RESEARCH INSTITUTE

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Approved By

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With a fresh look towards the harvest of prosperity and blessings ahead,

I seize each moment to reflect positively on my journey through

Traditional education to this summit.

Therefore, it is with immense thanks and invaluable appreciation

That I dedicate this work to the Lord,

Who by his abundant grace saw me through it all …

And to my loving family and teachers

Whose spiritual and emotional acumen also thrusted a deeper sense of inspiration, love and purpose in me.

DAIRY ENGINEERING SECTION
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CERTIFICATE

This is to certify that the thesis entitled, **"DEVELOPMENT OF MATHEMATICAL MODELS FOR THE PREDICTION OF SHELF LIFE OF CHEESE-*PURI* MIX",** submitted by **THANUJA D.** towards the partial fulfillment for the award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY ENGINEERING** of the **NATIONAL DAIRY RESEARCH INSTITUTE (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

MENON REKHA RAVINDRA
Major Advisor

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Date: /06/2011 *THANUJA D.*

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LIST OF ABBREVIATIONS

%	Per cent
°	Degree
μm	Micron(10^{-6})
MPa	Mega Pascal
wb	Wet basis
db	Dry basis
$R^{\bullet}$	Free radical
$RO^{\bullet}_2$	Peroxy radical
PV	Peroxide value
RH	Relative humidity
E	Relative deviation percent, %
a_w	Water activity fraction
M_o	Monolayer moisture content
cm	Centimetre (10^{-2})
LDPE	Low density poly ethylene
ml	Mililitre
TCA	Trichloro acetic acid
rpm	Revolution per minute
nm	Nano metre
OD	Optical density
kg	Kilogram
g	Gram
@	At
W_d	Dry weight of the powder

cm^3	Cubic centi metre
Y	Volume fraction of O_2 concentration
θ	Theta
R_y	Rate of oxygen absorbed
V	Headspace volume
$\alpha_{01}, \alpha_{02}, \alpha_{03}$	Regression constants
Vo_2	Amount of oxygen absorbed
Y_o	Initial O_2 concentration
$Y_{\theta s}$	Final O_2 concentration
A_p	Surface area of packaging material
Rh_s	Relative humidity of storage environment
P^*	Saturation vapour pressure of water at particular temperature
X_i	Initial moisture content
X_c	Critical moisture content
mEq	Miliequivalent
K_o	Oxygen permeability of packaging material
K	Water vapour permeability of packaging material
β	Greek letter beta
α	Greek letter alpha
γ	Greek letter gamma
Σ	Greek capital letter sigma
δ	Greek small letter delta
γ	Greek small letter gamma
Δ	Greek capital letter delta
O_2	oxygen

ABSTRACT

Predictive modeling of shelf life is a useful alternative to actual shelf life testing, since the latter is an expensive and time-consuming exercise, especially for dried foods. Mathematical models were developed to predict the shelf life of cheese – *puri* mix using two approaches, namely, deterioration due to moisture absorption and of lipid oxidation. Adsorption isotherms for cheese – *puri* mix were determined at 25, 35 and 45^{O}C using the gravimetric method with water activity ranging between 0.10 and 0.97. The sorption data was fitted to the GAB model using nonlinear regression analysis and the model described the sorption behavior adequately. Deterioration of the product due to absorption of moisture was anticipated to be manifested as non–enzymatic browning (NEB) and cakiness, while the index for oxidative rancidity was described in terms of peroxide value, which was expressed as a function of headspace oxygen concentration. NEB in the product was quantified in terms of its browning index and total colour difference. Development of cakiness in the sample was related to its sticky point temperature. The critical limits for moisture content (X_c) and headspace oxygen concentration (Y_f) for these deteriorative indices were also subjectively established at 45 ˚C and 95 % RH. Based on development of NEB, X_c was found to be 21.75 %, while for cakiness it was 25.3 %; Y_f for the product was determined as 14.3 %. The packaging material, low density polyethylene (350 gauge), was characterized for its water vapour transmission rate and oxygen permeability and found to be 5 $g.m^{-2}.day^{-1}$ and 1600 $cm^3.m^{-2}.day^{-1}$, respectively. The shelf life, at 38˚C and 95 % RH, was predicted to be 270 days based on moisture absorption, while it was predicted to be 180 days due to development of oxidative rancidity. Thus, the models predicted that the sample would spoil earlier due to lipid oxidation. The actual shelf life was determined to be 165 days; thereafter the sample was rejected due to rancidity development. Thus, the developed models showed a 90 % agreement with actual shelf life of cheese – *puri* mix, indicating the adequacy of the prediction.

सारांश

उत्पाद आयुकाल की अनुमानित रूप मॉडलिंग, वास्तविक आयुकाल परीक्षण के लिए एक उपयोगी विकल्प है, क्योंकि, विशेष रूप से सूखे खाद्य पदार्थों के लिए उत्तरार्द्ध परीक्षण एक महंगा और समय- खपत विधि है | चीज़ (cheese)-पुरी मिश्रण की शेल्फ लाइफ की पूर्वानुमान करने के लिए, दो दृष्टिकोण से, अर्थात्, आद्रता आमेलन और लिपिड ऑक्सीकरण के अवशोषण के कारण होने वाले गुणात्मक गिरावट का उपयोग कर गणितीय मॉडल विकसित किया गया | जल संक्रियता 0.10 एवं 0.97 मध्यक्रम युक्त ग्राविमेट्रिक प्रणाली का प्रयोग कर 25, 35 एवं 45 ^{o} सें पर चीज़ - पुरी मिश्रण के लिए संताप कल्पित रेखाओं का निर्धारण किया गया | अरेखीय प्रतीपगमन विश्लेषण का उपयोग करते हुए सोर्प्शन डेटा को GAB मॉडल पर लगाया गया और सोर्प्शन व्यवहार पर्याप्त रूप से वर्णित किया गया | आद्रता के अवशोषण के कारण उत्पाद की गुणात्मक गिरावट गैर - एंजाइमी भूरापन (NEB) और चिपचिपापन के रूप में प्रकट होना प्रत्याशित था, जबकि ओक्सिडेटिव दुर्गन्ध युक्त सूचकांक पेरोक्साइड मूल्य, जो हेड्स्पेस ऑक्सीजन कंसेन्ट्रेशन के एक क्रिया के रूप में व्यक्त किया गया था | उत्पाद में NEB भूरापन सूचकांक और कुल रंग अंतर की मात्रा पर निर्धारण किया गया | उत्पाद में चिप्चिपेपन का विकास इसके चिपचिपाहट बिंदु के तापमान के रूप में व्यक्त किया गया | आद्रता (X_C) और हेड्स्पेस ऑक्सीजन कंसेन्ट्रेशन (Y_f) के गंभीर सीमाएं, इन नाशक सूचकांकों के लिए, 45 ^{o}सें और RH 95% पर सम्वेदिक रूप से स्थापित किया गया | NEB के विकास के आधार पर, X_C के मूल्य 21.75% पाया गया, जबकि चिप्चिपेपन के लिए यह 25.3% था | उत्पाद के लिए, Y_f का मूल्य 14.3% निर्धारित किया गया | पैकेजिंग सामग्री, कम घनत्व पोलिएथिलिन (350 गेज), के पानी भाप संचरण दर और ऑक्सीजन पारगम्यता चिह्नित किया गया और वह 5 $g.m^{-2}.day^{-1}$और 1600 $cm^3.m^{-2}.day^{-1}$ पाया गया | चीज़ - पुरी मिश्रण की पूर्वनुमानिक शेल्फ लाइफ 38 ^{o}सें और 95% RH पर गणितीय मॉडल का उपयोग करते हुए विगणन किया गया | इसके मूल्य, आद्रता अवशोषण के आधार पर 270 दिन तथा ओक्सिडेटिव दुर्गन्ध के विकास के कारण 180 दिन पाया गया | इस प्रकार, मॉडल द्वारा अनुमान लगाया जा सकता है कि उत्पाद पहले लिपिड ऑक्सीकरण के कारण खराब होगा | वास्तविक आयुकाल परीक्षण से यह 165 दिन निर्धारित किया गया, उसके बाद उत्पाद में दुर्गन्ध होने के कारण उसे सम्वेदिक तौर पर अस्वीकार कर दिया गया | इस प्रकार विकसित मॉडल, चीज़ - पुरी मिश्रण के वास्तविक शेल्फ लाइ के साथ 90 % सहमति दिखाकर, इसके पूर्वानुमान की पर्याप्तता का संकेत दर्शाती है |

Chapter- 1

Introduction

Chapter 1
Introduction

Shelf life is the length of time that a product remains saleable without appreciable deterioration in its quality and acceptability. It is a critical post-processing parameter for any product. A food product looses its freshness and gets spoiled due to a variety of degradation mechanisms, the major of which results in the loss of colour, flavour, texture and nutritive value, ultimately affecting the product's shelf stability. Many factors influence the shelf life of a product and are broadly classified as intrinsic and extrinsic factors. Intrinsic factors are influenced by such variables as raw material type and quality, and product formulation and structure. Extrinsic factors are those factors the final product encounters as it moves through the food chain. This includes factors like microorganisms, package characteristics and environmental factors such as temperature, moisture, gas composition and light. The interactive effect of these factors on the quality of the product is usually manifested as physical, chemical and microbiological deterioration of the product and loss of its acceptability.

Determination of shelf life of a product aids in:

Selection of appropriate packaging material for a new product to provide desired shelf life at most economic cost.

Selection of alternate package for an already marketed product, to extend its shelf life or to reduce the cost by use of newer materials.

Declaration of "best before date" to assure the consumer of optimal quality and safety of the food.

Compliance with Government / Regulatory requirements regarding labeling

There are different methods of evaluating the shelf life of a product. Determining the shelf life of a food product is typically carried out by a process of informed trial and error, by keeping the product under controlled storage conditions until

spoilage is established by subjective and / or objective measurement. This is an expensive and time consuming exercise. The faster alternative to this conventional method of shelf life testing is by accelerated storage studies. The product is packed in the selected packaging material and exposed to more severe conditions of temperature and relative humidity, higher than the normal levels for conventional shelf life testing. These severe conditions accelerate the normal degradation rates, such that the product spoilage occurs significantly earlier. Data from a normal shelf life study on the same / similar product is then combined with the accelerated study data to project a normal-to-accelerated ratio of the estimated shelf life.

However, the accelerated conditions may trigger changes in the food that would not normally occur under conventional storage conditions. Also, the projected ratio of normal-to-accelerated storage does not always hold good, leading to erroneous projections of the shelf life of the product. In recent times, the onus has been on the use of mathematical models to predict the shelf life of a product. Such models look for statistical and mathematical relationships between three sets of variables: intrinsic (product - related); extrinsic (environmental factors) and implicit factors (behavior of the food under the influence of intrinsic and extrinsic factors). With increasing capabilities in computing, predictive modeling, especially in the area of microbial spoilage, has gained importance.

Puri is an unleavened wheat flour bread prepared by rolling dough balls to saucer – sized pancakes and frying in oil till it is puffed and golden brown. It is a very popular culinary preparation in the Indian subcontinent, served either as breakfast, snack or a light meal. Sometimes, the dough used for making *puri* is enriched using puree / paste of seasonal vegetables such as spinach, carrots, tomatoes etc or cooked legumes like green gram, Bengal gram etc. Cheese-*puri* mix is a dry, convenience product formulated from *maida* (refined wheat flour), cheddar cheese, ghee, skim milk powder (SMP) and permitted flavourings and preservatives.

Being a fat rich product, the product could be sensitive to lipid oxidation. It is also anticipated that moisture absorption by the product may result in Non-enzymatic browning, lumping, caking etc. The extent of these changes could be dependent on the extent of moisture / oxygen absorption by the product. Thus, the shelf life of the product could be influenced by the product properties, barrier properties of the packaging and the storage environment, which are independent factors, but have an interactive influence on the ultimate acceptability of the product. Therefore, it would be an area of interest to study the stability of this product under the influence of moisture permeation and oxygen absorption.

This work attempts to develop mathematical models to predict the shelf life of cheese- puri mix based on onset of deterioration due to moisture absorption and development of oxidative rancidity. The developed models will also be compared with real-time storage data for validation purposes. The specific objectives envisaged for the study are

- Development of mathematical models to predict the shelf life of cheese – *puri* mix based on onset of deterioration due to moisture absorption
- Development of mathematical models to predict the shelf life of the product based on onset of deterioration due to oxidative rancidity

Chapter- 2

Review of Literature

Chapter - 2
Review of literature

This chapter deals with the review of research work carried out by various workers relating to the main ingredients in cheese – *puri* mix. The literature related to mechanisms of spoilage and modelling of shelf life of food has also been reviewed.

2.1 Cheddar cheese

The major dairy ingredients of Cheese – *puri* mix include cheddar cheese and skim milk powder. Cheddar cheese is described as a relatively hard yellow to off-white, and sometimes sharp-tasting cheese originally made in the English village of Cheddar. Cheddar type cheeses are characterized by the mixing of salt with the curd before pressing it into a coherent loaf. Salt considerably retards the growth of lactic acid bacteria (Upadhyay, 2003).

2.1.1 Factors affecting cheese quality

2.1.1.1 Milk supply

The quality of the milk supply has a major impact on the quality of the resultant cheese. The three main aspects of quality that must be considered are the microbiological, enzymatic, and chemical quality of the raw milk used for cheese manufacture (Hickey et al., 2006)

2.1.1.2 Coagulant (Rennet)

The proportion of added rennet retained in cheese curd varies with rennet type, cook temperature, and pH at draining. Standardization of these variables is essential to produce consistent quality of cheese (Guinee and Wilkinson, 1992). Increased retention of the coagulant in the curd was found to result in greater initial hydrolysis of α_{s1} –casein, although it did not affect the sensory quality of cheese texture and flavour.

2.1.1.3 Starter

Starter strains play a key role in cheese manufacturing and ripening (Collins et al., 2003), the differences between the enzymes profiles of the starter strains affecting the cheese quality. Modern single- strain starters produce acid very reproducibly and if properly managed, show good phage resistance. Cheddar cheese is generally made using defined strains of *Lactococcus* as starter culture (McSweeney et al., 1994).

2.1.1.4 Cheese composition

The quality of cheese is influenced by its composition (Thakur et al, 1975), especially, moisture content, NaCl concentration (expressed as salt in moisture [S/M]), pH, moisture in non-fat substance (MNFS), and percentage fat in dry matter (FDM). The standards prescribed for premium cheddar cheese are pH, 4.95-5.10; S/M, 4.0-6.02%; MNFS, 52-56%; and FDM, 52-55%.

2.1.1.5 Ripening temperature

A known to influence the rate of ripening and cheese quality is the ripening temperature (Law et al., 1979). Ripening at elevated temperature is normally done with the objective of accelerating ripening, but it also affects cheese quality.

2.2 Skim Milk Powder

According to the PFA rules (1976) skim milk powder (SMP) is the product obtained from the skim milk of cow or buffalo or combination thereof, by the removal of water. It may contain added calcium chloride, citric acid and sodium citrate, sodium salts of orthophosphoric acid and polyphosphoric acid, not exceeding 0.3 % by weight of the finished product. Skim milk powder may not contain more than 1.5 % milk fat and moisture may not exceeded 5.0 %. The total acidity expressed as lactic acid should not exceed 1.5 %. The standard plate count should not exceed 50000/g. and the coli count must not exceed 90/g. the maximum solubility index should be 15.0 for a roller – dried and 2.0 for spray-dried product. (De, 1980)

2.2.1 Defects in skim milk powder

Some of the common defects and its probable causes reported in SMP are tabulated in Table 2.1.

Table 2.1 Some of the common defects and its probable causes reported in SMP

Name of the defect	Causes
A. Flavour	
Stale/old	Long storage, storage at high temperature, high temperature of heating, delayed cooling and removal of dried product from drying chamber and high moisture during storage.
Oxidized / Tallowy	Storage at high temperature, action of sunlight, high acidity in milk, metallic (copper & iron) contamination, high preheating temperature, delay in cooling of dried product from the chamber and higher oxygen content in the headspace.
Rancid	Low pre-heating temperature (lipase not activated).
Scorched / burnt	High temperature of drying, drum surface has pits and dull scraper knives (in drum dryer).
B. Body and texture	
Lumpy	Insufficient drying, absorption of moisture.
Caked	Absorption of moisture.
C. Colour and appearance	
Browning	Long storage, storage at high temperature, delayed cooling and removal of dried product from drying chamber, high moisture content during storage.
Scorched/burnt appearance	High temperature of drying, drum surface has pits and dull scraper knives (in drum dryer).
Lack of uniformity in appearance	Partial scorching during manufacture and partial discolouration after packaging.

Source: De, 1990

2.3 Water activity and sorption isotherm

The purpose of drying food products is to allow longer periods of storage with minimized packaging requirements and reduced shipping weights. The drying time, temperature, and water activity (a_w) influence the final product quality. Low temperatures generally have a positive influence on quality but require longer processing time. Low a_w retards the growth of micro-organisms, but results in higher lipid oxidation rates. Maillard browning reactions peak at intermediate a_w (0.6-0.7), indicating the need for a rapid transition from medium to high a_w (Franzen et al., 1990). Storage stability of a food product increases as a_w decreases, and the products that have been dried at lower temperatures exhibit good storage stability. Since lipid – containing foods are susceptible to lipid oxidation at low a_w; these foods must be stored in oxygen- impermeable packages (Franzen et al., 1990).

2.3.1 Definitions of water activity (a_w)

The water activity of a food or solution equals the ratio of the water vapour pressure of the food (p) to that of pure water (p_0) at the same temperature. When a solution becomes more concentrated, vapour pressure decreases and a_w drops from a maximum value of 1 for pure water (Heldman and Lund, 1992).

2.3.2 a_w calculation

a_w can be calculated according to the formula:

$$a_w = \frac{p}{p_o} \qquad (2.1)$$

Where p = vapour pressure of the food at T °C,

P_0= vapour pressure of pure water at T °C

2.3.3 Importance of a_w in food

Critical levels of a_W can be recognized above which undesirable deterioration of food occurs. Controlling a_W is the basis for preservation of dry and intermediate

moisture foods (IMF). Textural quality is also greatly affected by moisture content and water activity. Dry, crisp foods (e.g., potato chips, crackers) become texturally unacceptable upon gaining moisture above the 0.35 to 0.5 aw range (Katz and Labuza, 1981). IMFs-like dried fruits and bakery goods, upon losing moisture below 0.5 to 0.7 a_W become unacceptably hard (Kochhar and Rossel, 1982).

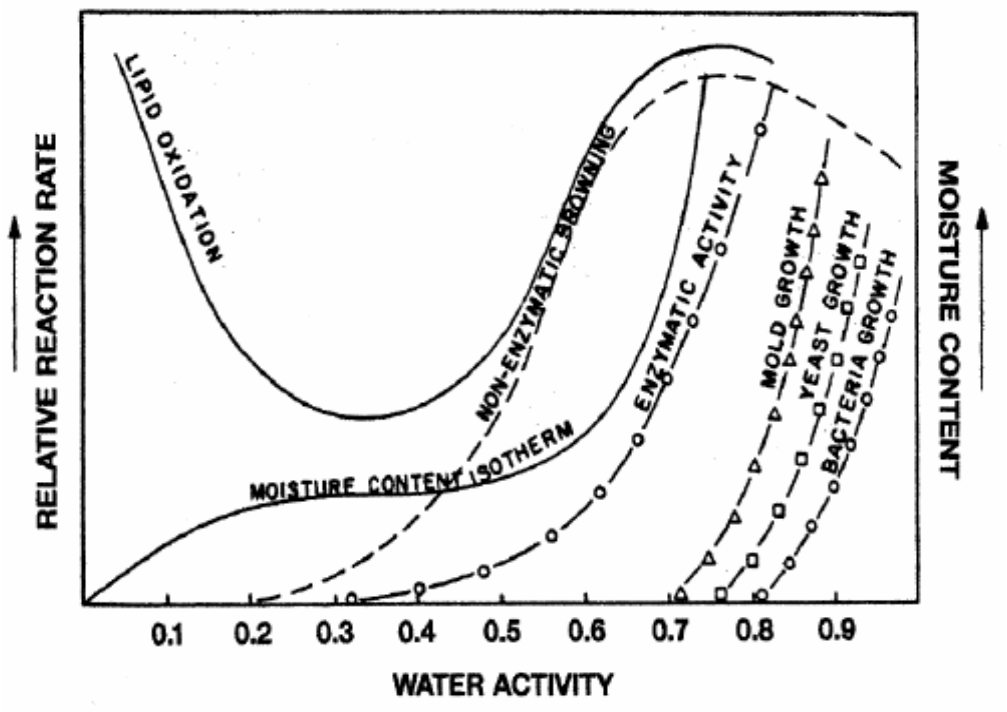

Fig. 2.1. Relationship between moisture content, water activity and relative reaction rate in food (source: Sewald and Devris, 2003)

Recrystallization phenomena of dry amorphous sugars caused by reaching an a_w of 0.35 to 0.4 affect texture and quality loss reaction rates, as already mentioned. Besides the specific critical a_w limits, a_w has a pronounced effect on chemical reactions (Fig. 2.1). This effect plays a very important role in the preservation of IMFs and dry foods. Generally, the ability of water to act as a solvent, reaction medium, and reactant increases with increasing a_w. As a result, many deteriorative reactions increase exponentially in rate with increasing a_w above the value corresponding to the monolayer moisture (Valents et al., 1997).

The critical a_w limits for microbial growth and the relative rates of reactions are important to food preservation such as lipid oxidation and non-enzymatic browning. Most reactions have minimal rates up to the monolayer value. Lipid oxidation shows the peculiarity of a minimum at the monolayer (M_0) with increased rates below and above it (Labuza, 1975; Quast et al., 1972). The proposed theories that attempt to explain the effect of a_w on food deterioration reaction as well as ways to systematically approach and model this effect are discussed by Labuza (1980).

Mathematical models that incorporate the effect of a_w as an additional parameter have been used for shelf-life predictions of moisture sensitive foods (Mizrahi et al., 1970; Cardoso and Labuza, 1983; Nakabayashi et al., 1980). Such predictions have an application in packaged foods in conjunction with moisture transfer models developed based on the properties of the food and the packaging materials (Taoukis et al., 1988).

2.3.4 Sorption isotherm studies

Moisture sorption isotherm describes the equilibrium relationship between moisture content and relative humidity of food product at a given temperature (Debnath et al., 2002).

The terms commonly associated with the moisture present in foods are:

Equilibrium moisture content: Moisture content of a product in equilibrium with the surrounding temperature and humidity condition.

Unbound moisture: Moisture in excess of the equilibrium moisture content corresponding to saturated humidity.

Bound moisture: Amount of moisture tightly bound to the food matrix with properties different from those of bulk water.

Free moisture content: Amount of moisture mechanically entrapped in the void spaces of the system, having nearly all properties similar to those of bulk water (Heldman and Lund, 1992).

When air is brought in contact with a wet food material, equilibrium between the air and food material is eventually reached. A plot of this equilibrium moisture content (EMC) v/s humidity at different temperatures is called the moisture sorption isotherm and is often used to illustrate the effect of temperature on this equilibrium relationship. Sorption isotherms are useful thermodynamic tools for determining the interactions between food and moisture (Debnath et al., 2002) and is important to predict the quality and stability of dehydrated foods (Vilades et al., 1995).

Sorption behavior of various food products has been extensively studied and a number of mathematical equations have been proposed (Table 2.2) to simulate the sorption behavior of foods.

Table 2.2. Mathematical models to describe sorption behavior

Model	Equation	Equation no
BET (Brunauer, Emmett Teller)	$M=\frac{M_0 c a_W}{[(1-a_W)+(c-1)(1-a_W)a_W]}$	2.2
GAB (Guggenheim-Anderson-deBoer)	$M=\frac{M_0 C K a_W}{[(1-K a_W)(1-K a_W+C K a_W)]}$	2.3
	$C = C_0 \exp\left\{\frac{\Delta H_g}{RT}\right\}$	2.4
	$K = K_0 \exp\left\{\frac{\Delta H_k}{RT}\right\}$	2.5
Chung-Pfost	$A_w = \exp\frac{-k}{RT\exp(-cM)}$	2.6
Halsey	$a_w = \exp\left[\frac{-k}{M^n}\right]$	2.7
Henderson	$1-a_w = \exp(kTM^n)$	2.8
Oswin	$M = k\left(\frac{a_W}{1-a_W}\right)^n$	2.9

Source: Hui (2006)

Five types of isotherms (Fig. 2.2) are described by Brunauer et al., (1940). Type 1 is the well known Langmuir isotherm, obtained by the monomolecular adsorption of gas by porous solids in a finite volume of voids. Type 2, called the sigmoid isotherm, which is obtained for soluble products and shows an asymptotic trend as water activity tends towards 1.

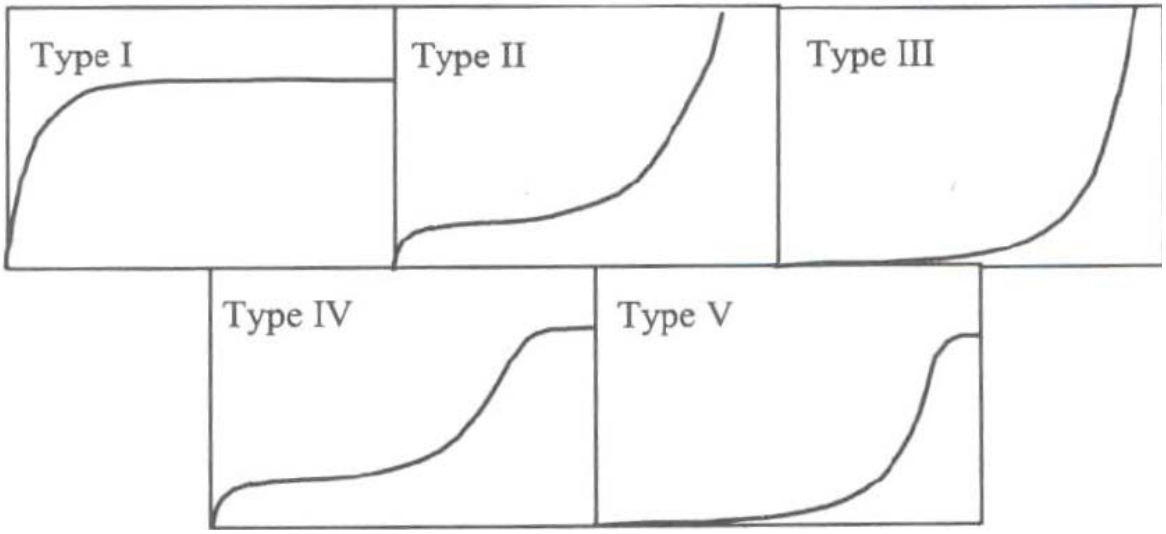

Fig. 2.2. Different types of Sorption isotherms

Type 3, known as the Flory-Huggins isotherm, accounts for the adsorption of a solvent or plasticizer like glycerol, for example, above the glass transition temperature. The Type 4 isotherm describes the adsorption by a swellable hydrophilic solid until a maximum of hydration of sites is reached. Type 5 is the B.E.T. multilayer adsorption isotherm, observed for the adsorption of water vapour on charcoal and related to types 2 and 3 isotherms. The two isotherms most frequently found for food products are Types 2 and 4 isotherms (Mathlouthi and Roge, 2003).

2.4 Non enzymatic browning

Nonenzymatic browning (NEB) via the Maillard reaction is an important mode of deterioration in dried dairy powders, which limits shelf life (Saltmarch et al., 1981). Dairy foods containing lactose are readily susceptible to NEB. The first step in the Maillard reaction involves the nucleophilic attack by the nitrogen atom of an amino compound on the electrophilic carbonyl group of an aldehyde or

ketone; in food systems, the reactants are predominantly proteins and reducing sugars (Kaneko et al., 1991). In the presence of moisture, these components readily participate in Maillard reactions. The Maillard reaction is affected by the concentration of the initial reactant species, pH, water content, and presence of substances such as humectants and bisulfite (Franzen et al., 1990). Some physical factors, such as processing and storage temperature, atmospheric oxygen, and packaging during storage can also affect the Maillard reaction in foods. The deleterious effects of non-enzymatic browning include: decreased nutritional value from protein loss, off-flavor development, undesirable color, decreased solubility, texture changes, destruction of vitamins, and increased acidity (Saltmarch et al., 1981). Brown pigment formation has been used as an indicator of the Maillard reaction in food (Saltmarch et al., 1981).

2.5 Sticky point of foods

A widely accepted definition of sticky point temperature is based on the fact that for given combination of material temperature and moisture content, the mass of a powdery material resists movement and is no longer free- flowing (Kudra, 2003). In moisture content-temperature coordinates; the set of sticky point temperature forms the so called sticky point curve, which provides a sharp boundary between the sticky region below the curve, and non- sticky region above the curve. The sticky point temperature decreases with moisture content and for some powdery materials it can drop below 40˚C e.g, tomato (Lazar et al., 1956), reaching even 20˚C, as in the case of sucrose- fructose mixture (Downton et al., 1982).

With such definition, stickiness reflects mostly cohesive forces between individual particles in the bulk of solids. Genskov (1990) considered the sticky- point as a special case of cohesion that is particularly relevant to drying as it links material moisture content with temperature. Of the variety of methods used to quantify cohesion and adhesion of powders, the stirrer- type devices have been found to be the best for measurements of the sticky - point temperature (Pasley et al.,

1995). These devices exploit a definition of the sticky- point temperature as the temperature at which the force necessary to turn an impeller in a material sample with defined moisture content increases dramatically. In the original design by Lazar et al., (1956) the impeller was periodically turned by hand with simultaneous raising powder temperature. A sharp increase in the force required to turn stirrer indicated the sticky point temperature. Another method to measure the sticky point temperature measurement involved placing a specific amount of powder on a soft plate and gradually heating the plate, the temperature at which the particles began to exhibit visible cohesion was recorded as the sticky point temperature (Chegini and Ghobadian, 2007).

2.6 Lipid oxidation

Lipid oxidation is one of the most basic chemical reactions that occur in food; generally resulting in deterioration of sensory and nutritional quality. It is essentially a free radical chain reaction involving initiation, propagation, and termination stages. Unsaturated fatty acids are oxidized to form odourless, tasteless hydro peroxides. These are unstable and degrade to yield flavourful carbonyls and other compounds (Fox and McSweeney, 2006).

2.6.1 Mechanism of lipid oxidation

The fundamental principles were elucidated by the work of Farmer et al., (1942), Bolland and Gee (1946) and Bateman et al., (1953). A sequence involving initiation, propagation and termination reactions has been proposed (Ozligen and Ozligen, 1990) and is as given in Fig. 2.3.

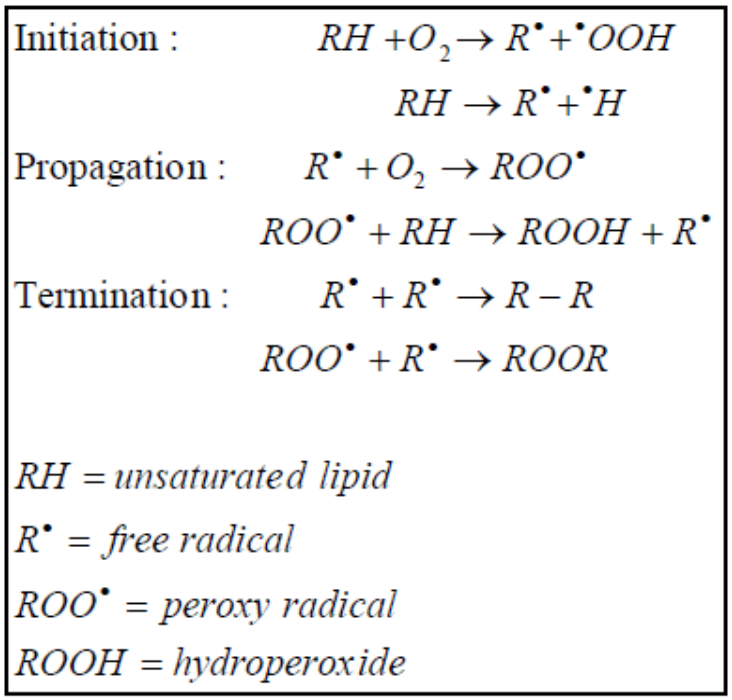

Fig. 2.3. Mechanism of lipid oxidation

The initial step in the autoxidation of unsaturated fatty acids involves the formation of free radicals. The formation of the initial free radical to start the oxidation process may be due to factors such as irradiation, metal complexes and enzymes or active oxygen species. In the case of monounsaturated and non-conjugated polyunsaturated fatty acids in milk lipids, the reaction is usually initiated by removal of hydrogen from the methylene group adjacent to the double bond. The resulting free radical reacts with ground- state molecular oxygen to form a peroxide free radical. This, in turn reacts with another unsaturated molecule to continue the chain reaction and generate a hydroperoxide.

Schaich (1980) reported that the rate of oxidation is directly proportional to the amount of peroxide produced and at low oxygen pressures to the concentration of oxygen.

2.6.2 Oxidation products of off-flavour

Autoxidation of unsaturated fatty acids gives rise to unstable hydroperoxides, which decompose to a wide range of carbonyl products. Many of which can contribute to off-flavours in dairy products. The principal decomposition products of hydroperoxides are saturated and unsaturated aldehydes (Frankel et al., 1961) with lesser amounts of unsaturated ketones (Stark and Forss, 1962), saturated and unsaturated hydrocarbons (Forss et al., 1967), semialdehydes (Frankel et al., 1961) and saturated and unsaturated alcohols (Hoffman,1962; Stark and Forss,1966).

Forss et al (1955a, b) reported that n-hexanal, 2-octenal, 2-nonenal, 2, 4-heptadienal and 2,4-nonadienal are the principal carbonyls contributing to the copper-induced “cardboard” off-flavour in milk. Drier flavour in foam spray-dried milk has been associated with 6-trans-nonenal, which has a flavour threshold in fresh milk of 0.07 $\mu g.kg^{-1}$ (Parks et al., 1969). Bassette and Keeney (1960) implicated a homologous series of autooxidation-derived saturated aldehydes, together with products of maillard reaction, in “cereal-type” off-flavours in powdered skim milk.

2.6.3 Measurement of lipid oxidation

The extent of oxidation in foods is of importance to predict the quality and stability of the product. Many analytical methods have been reported in literature for the measurement of the extent of lipid oxidation of food products. Theses involve the estimation of peroxide value (PV), 2 – thiobarbituric acid (TBA) value, Anisidine value and an ultraviolet method for determination of conjugated dienes (Rossell, 1989)

2.7 Permeability of Packaging Material

The permeability of the packaging material is one of the most critical features of the package for affecting the quality of the food product. According to ASTM (1994), transmission rate is defined as the movement of a permeant in unit time

through a unit area under specified conditions of temperature and relative humidity. Permeability is defined as the movement of a permeant through a unit area of unit thickness induced by a unit vapor pressure difference between two specific surfaces under specific conditions of temperature and humidity at each surface.

2.7.1 Measurement of water vapor permeability of flexible packaging material

Of the various techniques available for the measurement of water vapor permeability of flexible packaging materials, the most frequently used is the gravimetric method (Troller and Christian, 1978). In this method, a circular sample of film is sealed with wax across the opening of a dish, usually of aluminum, containing a desiccant. The assembly is stored under conditions of constant temperature and relative humidity and the moisture uptake by the desiccant was determined by weighing the dish assembly at intervals. This method is simple, inexpensive, but very slow. Other procedures for measuring water vapor permeability include use of radioactive water, electric hygrometers, electrolytic moisture meters, infrared absorption, and thermal conductivity and gas chromatography.

2.7.2 Measurement of oxygen permeability of flexible packaging material

A number of studies have been carried out to comprehend the effects of various factors on gas transmission rate through a flexible packaging material. Peterson (1968) developed a simple gas permeability apparatus, which performed all the essential functions of a complex conventional design. The apparatus measured gas transmission rates of variety of polymer films quite accurately. However, considerably high pressure gradients were employed for the gas transmission rate measurement. Rigg (1979) modified the ASTM method (D-1434) to enable permeability measurement at various relative humidities. He observed a marked increase in the oxygen permeability of nylon and cellophane films above 70% RH

and recommended that the permeability of hydrophilic materials used for packaging, should be measured at 100% RH.

2.8 Prediction of shelf life

Shelf life studies can provide important information to ensure the consumers of a high quality product for a significant period of time after production. Shelf life determination may also be required when there are changes in product design, food formulation, and package or storage system.

Shelf life simulation of food products has been studied using models based on the deterioration of food products as a function of storage temperature (Kwolek and Bookwalter, 1971; Iglesias et al., 1979). Some works have also reported the shelf life of moisture-sensitive foods as a function of more than one environmental factor (Azanha and Faria, 2005). Cardoso and Labuza (1983) used Arrhenius relationship-based mathematical model to predict shelf life of a packaged pasta product as a function of temperature and relative humidity. Pieglovanni et al. (1995) developed a more general model based on the product's water activity and the water vapour transmission rate of plastic films at different temperatures and RH using ASTM dynamic and gravimetric methods. Composite algorithms for predicting the rate of oxygen uptake and loss of shelf life of dry foods based on basic deteriorative mechanisms and kinetics of food deterioration were successfully applied for roasted and ground coffee (Cardelli and Labuza, 2007). Gomez – Alonso et al., (2004) modeled the shelf life of olive oil triacylglycerols based on kinetics of oxidative rancidity and established a linear relationship between rancidity threshold limits established for peroxide value and the sensory stability of the product.

Chapter- 3

Materials and Methods

Chapter - 3
Materials and methods

This chapter deals with the methods and techniques followed to fulfil the various objectives envisaged for this study. It includes the raw materials used for the study, analysis of samples, model development and validation for predicting the shelf life of cheese - *puri* mix.

3.1 Raw materials

The various raw materials and its proportion used for the preparation of cheese - *puri* mix is outlined in Table 3.1. Cheddar cheese, ripened for one month, taken from the experimental dairy at NDRI, Bangalore was used for the preparation of the mix. Similarly, the *ghee* used for preparation of the mix was freshly prepared at the experimental dairy. Skim milk powder (Brand: Nandini), *maida*, chilli powder, salt, soda, citric acid and *jeera* was sourced from the local market. Care was exercised to ensure that fresh and good quality material was used as the ingredients in the mix.

Table 3.1. List of ingredients used for the preparation of cheese – *puri* mix

Cheddar cheese	**1 kg**
Ghee	**350 ml**
Maida	**3 kg**
SMP	**1 kg**
Chilli powder	**50 gm**
Salt	**60 gm**
Soda	**10 gm**
Citric acid	**10 gm**
Jeera	**35 gm**

3.2 Preparation of Cheese – *puri* mix

The mix was prepared as per the method standardised at the Dairy Technology section of NDRI, Bangalore. The flow chart of the method is presented in Fig.3.1.

Grate the cheese

↓

Add maida, SMP, soda, citric acid, ghee & mix properly

↓

Seive the mix

↓

Add chilli powder, salt, and sieve again

↓

Add jeera

↓

Dry the mix in a tray dryer at 50-52ºC, 23 h

↓

Cheese – *puri* mix

Fig. 3.1. Flow chart for preparation of Cheese – *puri* mix

3.3 Analysis of sample

The mix prepared as per Fig. 3.1.was used for analysis and experiments in this study.

3.3.1 Moisture

The moisture content and total solids of the mix was determined by the method detailed in IS (SP: 18, part XI, 1981). About 5g of the sample was accurately weighed in to a (previously dried & weighed) flat bottomed glass dish. The dish containing the material was heated in an oven maintained at 98 ±2˚C for about 5h. The dish was the cooled in a desiccator and weighed. This process of drying, cooling and weighing was repeated at 30 min intervals until the difference

between two consecutive weighing was less than 1mg. The final mass was recorded and the % moisture was calculated as

$$\% \text{ Moisture content} = 100\frac{w_1 - w_2}{w_1 - w} \quad (3.1)$$

W_1- Weight in g of the dish with material before drying

W_2 - Weight in g of the dish with material after drying

W- Weight in g of empty dish.

3.3.2 Fat

The fat content in cheese - *puri* mix was estimated using Mojonnier fat extraction apparatus according to the method detailed in IS (SP: 18, part XI, 1981).

Well mixed sample of about 2 g was weighed in to a small beaker. 10 ml of concentrated HCl was added to this sample and the contents were mixed properly. The beaker was now transferred to a boiling water bath to digest the mixture. After digestion, the contents were cooled and carefully transferred in to a Mojonnier fat extraction tube. Alcohol (95% v/v) of 10 ml was then added in to the tube, stoppered and shaken vigorously for about 1.5 min. Alcohol was added as it prevents the formation of jelly or colloid like substance which always forms when ethyl ether is added without alcohol. The contents were then mixed well.

This was followed by the addition of 25 ml of diethyl ether (to dissolves the fat), after which the tube was again stoppered and shaken vigorously for about 1.5 min. Next 25 ml of petroleum ether (BP: 30 - 60 ˚C) was added & the contents mixed for 1.5 min. Petroleum ether takes out the last traces of moisture from ethyl ether extract and aids in the extraction of fat. The tube was allowed to stand until the ether layer was clear & completely separated from the aqueous layer, which took about 30 min. The ether mixture containing the extracted fat was then carefully decanted into a fat glass dish which had been previously dried & weighed. A second and third extraction was repeated by addition of 15 ml of diethyl ether and petroleum ether each.

The dish containing the extracted fat was placed on a boiling water bath to evaporate the solvent. The finish – drying of the solvent was carried out in a hot air oven at 100 ˚C for 3 hr. The dish was then cooled in desciccator and the final weight was taken. The % of fat in the sample was calculated as per equation (3.2).

$$\% \text{ Fat} = 100\frac{w_2 - w_1}{w} \qquad (3.2)$$

W_2 – Weight in g of dish with extracted fat after dying

W_1 – Empty weight in g of the dish

W – Weight in g of sample

3.3.3 Protein

The protein content of the mix was determined by Boric acid method using Gerhardt Kjeldahl instrument.

0.2 g of well mixed sample (accurately weighed) was transferred to the Kjeldahl tube. Then 5-10 ml of distilled water, 12.5 ml of concentrated sulphuric acid and a digestion mixture comprising of 0.2 g of copper sulphate (catalyst) along with 10 g of potassium sulphate (which enables the temperature of boiling mixture to be raised) was added to the sample. The tube was placed in the digestion unit and its temperature was gradually increased to 300 ˚C. The mixture was boiled till the digestion was complete (indicated by pale green colour).

25ml of 2% boric acid solution was taken in 250 ml conical flask and 2 - 3 drops of mixed indicator (0.1 % of methylene blue in ethyl alcohol 95% by v/v) was added to it. The conical flask containing 2% boric acid solution was placed in the cabin of the distillation unit, such that, the tip of the tube through which the ammonia came out was completely dipped in the solution. Digested sample in the tube was also fixed to Kjeldahl plus distillation unit. 50 % alkali (NaOH) was added, by pressing the alkali pump, till the digested sample turned to brownish blue colour. The distillate was collected in to the 250ml conical flask containing 2 % boric acid by pressing the process button. Colour change in the distillate was

observed and it was tested for complete distillation of ammonia using a litmus paper.

The contents in the conical flask were titrated against 0.1N sulphuric acid. Blank determination was carried out using 0.05g of pure sucrose in the place of sample. The titre values for sample and blank were recorded and % protein was calculated using equation (3.3).

$$\% \text{ Protein} = \frac{1.4(S-B)0.1}{w} \times 6.38 \qquad (3.3)$$

S - Titre value for sample

B - Titre value for blank

W - Weight of in g of sample taken

3.3.4 Lactose

Lactose content in the product was determined by the volumetric method of Lane-Eynon (IS: SP 18, part XI, 1981) prescribed for milk.

10 g of powder was dissolved in 100 ml of distilled water. 25 ml of the reconstituted mix was taken in a 250 ml volumetric flask and to this 150 ml of distilled water and 5 ml dilute ammonia solution was added. After proper mixing, it was allowed to stand for 15 min. The exact equivalent of dilute acetic acid to neutralize the ammonia was added and mixed. To this solution, about 12.5 ml of zinc acetate solution was added, followed by 12.5 ml of potassium ferrocyanide solution. The contents were mixed and made up to 250ml. After the precipitate settled well, the solution was filtered. The filtrate was filled in a burette; 5ml of each of Fehling A and Fehling B solution was taken in a conical flask and boiled on low heat. The boiling solution was titrated against the filtrate taken in the burette using methylene blue as indicator and the titre value was recorded. Titration was also carried out with standard lactose solution (0.5%) in the same

manner as above. Lactose content of the sample was computed using equation (3.4).

$$\% \text{ Lactose} = \frac{W \times 250 \times 100}{V \times W_1} \quad (3.4)$$

W- Weight in mg of lactose required to reduce 10 ml of Fehling solution

V- Volume in ml of filtrate required to reduce 10 ml of Fehling solution

W_1- Weight in g of sample taken for analysis

3.3.5 Ash

Approximately 5 g of the cheese – *puri* was taken in a silica dish and ignited in a muffle furnace at a temperature of 550 ± 10˚C till it was carbon free (no black particles were observed). The dish was cooled in a desciccator and weighed. Ash content was calculated as follows.

$$\% \text{ Ash} = (100 * W_1) / W \quad (3.5)$$

W_1 - Weight in g of ash

W - Weight in g of sample taken

3.3.6 Total carbohydrates excluding lactose

The total carbohydrate content of the sample, excluding lactose content, was estimated using the equation 3.6.

$$\text{T.C.} = 100 - (M + F + P + A + L) \quad (3.6)$$

T.C. – Total carbohydrates excluding lactose

M - % Moisture of the sample

F - % Fat of the sample

P - % Protein of the sample

A- % Ash of the sample

L - % Lactose of the sample

3.3.7 Water activity

The water activity of product was measured using a digital water activity meter (Rotronic hygroplus, Model WA 1).

The equipment was switched on and warmed up for 15 min before measurement of water activity. Powdered sample was filled in the sample cups up to half marked level and positioned below the sensor probe inside the holder. Both temperature and a_W reading was noted down from the display unit, when the arrows on either side were equalised.

3.3 Sorption Isotherm Studies

3.3.1 Measurement of sorption equilibrium:

Equilibrium moisture content (EMC) was obtained during adsorption of moisture by the cheese - *puri* mix. Eight saturated salt solutions (LiCl, $MgCl_2.6H_2O$, K_2CO_3, Mg $(NO_3)_2.6H_2O$, NaCl, KCl, KNO_3, K_2SO_3) giving relative humidities of 0.113, 0.329, 0.333, 0.536 0.762, 0.855, 0.93 0.97 respectively at 25˚C were used.

Each solution was transferred to airtight desiccators. Duplicate samples containing about 1 g of cheese - *puri* mix were weighed in small beakers. Potassium sorbate (0.1 mg) was added to each sample to prevent the microbial growth. The sample was evenly spread throughout the beaker using a glass rod; the beakers were then placed above the saturated solutions inside the desiccators which were then tightly closed. The desiccators containing the samples were placed inside an incubator maintained at a particular temperature; three temperatures 25˚C, 35˚C and 45˚C were used for the study. Since the equilibrium relative humidity values for saturated salt solutions vary with temperature, they were estimated from the equations (Appendix – 1) developed by Labuza (1984).

Mass of each sample was measured with a first interval of 20 days and thereafter every week. The duration for removal, weighing and replacing the samples was less than 10 seconds. This minimized the atmospheric moisture sorption during weighing. Experiments were completed when less than 1% weight change was observed between two readings. Equilibrium moisture content was calculated by static moisture gain / loss by/from the test samples.

3.3.2 Mathematical modelling of isotherms

Equilibrium moisture content and equilibrium relative humidity data was fitted using Guggenheim-Anderson-deBoer (GAB) model. The three models are represented in Table 2.2. In this models, X and M_0 represents equilibrium and monolayer moisture contents of cheese - *puri* mix (kg of water solid^{-1}), a_W is the water activity (fraction), C and K are GAB model constants**.**

The constants of selected models were estimated by non-linear regression equation using software package (Systat 8.0). Goodness of fit of models was judged from relative deviation percent E and average residuals (Eqn.3.7).

$$E = \frac{100}{N}\sum_{1}^{N}\frac{\left|\left(R_{exp} - R_{pre}\right)\right|}{R_{exp}} \qquad (3.7)$$

Where, *E* is the mean relative deviation modulus in %, *N* is the number of data points, R_{exp} is the experimental value and R_{pre} is the predicted value (Das, H., 2005).

3.4 Accelerated storage of cheese - *puri* mix;

About 100 g of cheese - *puri* mix was packed in each of 10 LDPE pouches of size 18*15.5 cm. Thickness of the pouch was 350 gauge (87.5 μ m). The pouches containing powder were kept in a thermostatically controlled incubator maintained at 45 ± 1˚C temperature and 95 ± 2% relative humidity. Temperature inside the incubator was maintained by using a contact thermometer type temperature sensor-controller and relative humidity was maintained using saturated potassium sulphate solution. Every 7 days, one of the pouches was taken out of the controlled environment and its contents was analysed for sensory characteristics (colour and flavour), moisture content, browning and peroxide value.

3.4.1 Measurement of Non-enzymatic browning

One of the deteriorative changes anticipated during storage of cheese – *puri* mix due to moisture migration into the product was non–enzymatic browning. The same was quantified during the study by estimation of browning index and total difference in colour using image analysis.

3.4.1.1 Browning index

Browning index of the powder was measured using an enzymatic digestion method (Palombo et al, 1984, Franzen et al., 1990) which releases the brown pigments. 2 g of well mixed sample stored under accelerated storage condition was reconstituted with 10 ml of distilled water at 35˚C and 3ml of the mixture was transferred in to tubes containing 0.8ml of pronase solution (contained 10 mg pronase protease Steptomyces griseus: CALBIOCHEM; activity 61676.6 PUK/g) per ml of Tris buffer (standard Tris buffer solution: CALBIOCHEM; molecular weight of 121.1 g, pH 7.3, 0.2M HCl, and distilled water (1:1:2), pH 7.00). The tubes were incubated at 35˚C for 180 min and then cooled in an ice bath. After addition of 0.3 ml of 100 % TCA (Trichloro Acetic acid) , the mixture was centrifuged at 3700 rpm for 20 min and the contents filtered through a filter paper (Whatman No. 1). The absorbance of the clear solutions were measured at 320nm (A_{320}) and 550nm (A_{550}) using a spectrophotometer (ANTHELIE light2, Secoman France). The reading at 550nm was used for correction due to turbidity and the browning index was expressed in terms of the optical density (OD = A_{320} - A_{550}) g of solid material^{-1}. The product was sampled at regular interval of 7 days and tested for its browning index.

3.4.1.2 Total colour difference

The colour of the product during storage in terms of its L, a, b values were recorded using the histogram window method of Adobe Photoshop (Adobe Systems 2002) as described in Yam and Papadakis (2004). The product was evenly spread (2.5g in 1 square) in a petridish with the help of a spatula to obtain a uniform surface; care was taken to avoid air pockets. The sample was placed

on the surface of a flat bed scanner (HP Scanjet 5370C) and the product images were scanned. The scanned images were imported to be read in to the photoshop software. The Lightness, a and b values were then read from the histogram window and converted to the standard L*, a* and b* values using following formulae (Yam and Papadakis 2004)

$$L^* = \frac{100\,L}{255} \qquad (3.8)$$

$$a^* = \frac{240\,a}{255} - 120 \qquad (3.9)$$

$$b^* = \frac{240\,b}{255} - 120 \qquad (3.10)$$

The total colour difference (E) (Rhim *et al.*, 1989) were calculated as follows.

$$\Delta E = [(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2]^{0.5} \qquad (3.11)$$

The subscript 'o' refers to the control (freshly prepared) sample values.

3.4.2 Sticky point temperature

Moisture migration into the product could also lead to development of lumpiness or cakiness in the cheese – *puri* mix. The effect of moisture on this characteristic of the product was quantified in terms of its sticky point temperature. Based on the review of literature reported under section 2.5, sticky point temperature for this study was measured based on the principle that the onset of stickiness in a product could be indicated by a sudden change in torque during shearing of the sample (Lazar et al. 1956).

50 g of the sample was taken in a glass beaker and calculated amounts of water were added to the powder to achieve predetermined levels of moisture content in the sample. The sample was mixed properly for uniform distribution of water and allowed to equilibrate at least for an hour prior to analysis. The sample was heated to different temperatures by placing the beakers (containing the equilibrated sample at each moisture content) in a water bath set at the required temperature. Before each experiment to record sticky point temperature, a

representative sample was drawn from the beaker to measure its moisture content as described in section 3.3.1.

The sample was sheared using a Brookfield viscometer (Model- D220 Spindle type-RV6) and the torque was monitored. For any given moisture content, the temperature of the sample at which a sudden increase in torque was observed was noted as its sticky point temperature. Three replications were carried out to determine the average sticky point temperature at each moisture level.

3.4.3 Measurement of peroxide Value

During storage of the product, lipid oxidation and rancidity development was the other spoilage that was anticipated for cheese – *puri* mix. This change was quantified in this study in terms of the peroxide value of cheese puri mix, measured as per the standard method prescribed in Kirk and Ronald (1991)

Fat was extracted from the cheese - *puri* mix using Soxhlet apparatus. About 1 g of extracted fat was taken in a 100 ml iodine flask. About 20 ml of solvent mixture (acetic acid: chloroform at 2:1) was added to the fat. The flask was swirled to dissolve the fat and mixed with 1 g of potassium iodide. The flask was stoppered and shaken for one minute and then placed over a boiling water bath until the first boiling of the contents were observed in the flask. The contents was cooled and added with 25 ml distilled water. It was then titrated against 0.002N sodium thiosulphate using 1 % starch as indicator; the end point was indicted when the upper layer of the solution was clear. Blank analysis was done using the same procedure described above without adding any fat. Peroxide value (PV) (m Eq.kg^{-1}) of the sample was calculated using equation (3.12).

$$PV = \frac{(V - V_0)N \times 1000}{W} \qquad (3.12)$$

V - ml of titre value for sample
V_0 – ml of titre value for the blank
N – Normality of sodium thiosulphate
W – weight in g of extracted fat

3.5 Rate of oxygen absorption

In order to develop a model for the oxygen absorption by cheese - *puri* mix, a set of experiments were conducted. In these experiments, 5 g of cheese - *puri* mix was kept inside a 40 ml plastic bottle. The bottle was sealed using a silicon tape and placed inside an incubator where the environment was maintained at 38 ± 1 ^{o}C. Seven such bottles were used for study. One bottle was removed at a time interval of 7 days and the product contained in it was analysed for its moisture content, free fat content and peroxide value. Initial and final oxygen in the headspace of the bottles were measured using a digital gas analyser (PBI Dansensor checkmate II, Denmark). For analysing the oxygen concentration, a small volume of gas sample was drawn out by inserting a hypodermic needle in to the bottle headspace through a septum attached to the bottle.

The rate of oxygen absorption was expressed as a function of the oxygen concentration in the headspace of the container. The model development is as described below. If Y_i (volume fraction) is the concentration of oxygen after duration of θ_i (day) of placing the powder inside the bottle and the O_2 concentration has been changed to Y_{i+1} at time θ_{i+1}., the rate of oxygen absorption R_y (cm^3.kg dry powder^{-1}.day^{-1}) by the powder can be expressed by the equation (3.13).

$$R_y = \frac{V}{W_d}\frac{Y_i - Y_{i+1}}{\theta_i - \theta_{i+1}} \qquad (3.13)$$

Where, W_d (kg) is the dry weight of the powder kept inside the plastic bottle and V (cm^3) is the headspace volume left after placing the sample.

The average values of oxygen concentration Y (volume fraction) inside the container during the period (θ_{i+1}- θ_i) when the above rate R_y takes place could be approximated as

$$Y = \frac{Y_i - Y_{i+1}}{2} \qquad (3.14)$$

Using the experimental data of oxygen concentration at different time intervals within the container, values of Y and R_y were computed. The rate of oxygen absorption R_y was expressed as a function of Y by a second order regression equation (3.15) using Systat 8.0 software package.

$$R_y = a_{01} + a_{02}Y + a_{03}Y^2 \qquad (3.15)$$

Where, a_{01}, a_{02}, and a_{03} are the regression constants.

Goodness of fit of the regression model was tested by computing the relative deviation percent.

3.6 Amount of oxygen absorbed and peroxide value

The amount of the oxygen absorbed by the cheese - *puri* mix contained inside the plastic bottles during certain duration of time θ_s can be obtained from the equation (3.16).

$$V_{O_2} = \frac{\left[V\left(Y_0 - Y_{\theta S}\right)\right]}{W_d} \qquad (3.16)$$

Where, V_{O_2}(cm^3.kg dry powder $^{-1}$) is the amount of oxygen absorbed during time interval θ_s (day), W_d (kg) is the dry weight of the powder kept inside the plastic bottles and V_e(cm^3) is the headspace volume of the bottles, Y_0 and Y_{θ_S} (volume fraction) are the initial and final concentrations of oxygen present inside the bottle, respectively, after time duration of θ_S (day) respectively.

A relation between the amount of oxygen absorbed V_{O_2} (cm^3.kg dry powder $^{-1}$) and peroxide value (mEq O_2.kg fat^{-1}) was developed by fitting the experimental data of peroxide value (PV) and V_{O_2}

$$PV = f\,(V_{O_2}) \qquad (3.17)$$

The developed relationship was analyzed for its accuracy based on relative deviation percent. The developed models for rate of oxygen absorption and

peroxide value were used for shelf life prediction of cheese - *puri* mix stored inside the pouches.

3.7 Measurement of water vapour permeability of packaging material

Water vapour permeability of films was determined at 38±2˚C using ASTM E96 method. A known quantity of calcium chloride was placed inside the aluminium cup and its mouth (50 cm^2) was sealed with the packaging material being evaluated. Care was taken that added calcium chloride should not touch the film it may cause the damage to the film. Cups were stored in an incubator containing a saturated solution of potassium nitrate to maintain a RH of 90±2%. Weight gained by the cups recorded every alternative day. Experiments were completed when less than 1% change in weight was observed between two readings. Water vapour permeability was calculated using the following equation.

$$WVTR = \frac{\Delta W \times A}{\Delta t} \qquad (3.18)$$

Where, Δ W = weight gain, g

A = area of packaging material, m^2

Δ t = Time elapsed, days

3.8 Measurement of oxygen permeability of packaging material

Oxygen permeability of packaging material was measured using the standard method prescribed in ASTM D1434 (volumetric method) using the Gas Permeability Cell System (Custom Scientific Instruments Inc, Pennsylvania)

A sample of the LDPE film was mounted between the test cells and the measurement of the rate of permeation through LDPE film determined as the change in volume of permeated gas, under constant pressure differential across the film specimen. The change in the volume of the permeation was measured as a function of time by following the displacement of a short column of coloured liquid (mercury) in a glass capillary. Gas transmission rate was calculated from the given formula.

$$K_o = \frac{\Delta v \times \delta \times 60 \times 24}{A \delta P} \qquad (3.19)$$

Δv- rate of change in volume of liquid in capillary, cm^3/ s
A- Area of packaging material, m^2
δ – Thickness of the film, m
Δp- Pressure difference, psi

3.9 Prediction of cheese - *puri* mix shelf life

3.9.1 Moisture gain and storage life prediction

The model developed for prediction of shelf life due to moisture absorption is described below.

The rate of change of moisture content $dX/d\theta$ of cheese puri mix with time of storage can be expressed as

$$\frac{dX}{d\theta} = W_d K A_p (Rh_s P^* - a_w P^*) \qquad (3.20)$$

Where, W_d (kg) is the dry weight of powder kept inside the pouch, P* (Pa) is the saturation vapour pressure of water at temperature T_a (˚C) of storage, R_h (fraction) is the relative humidity of the storage environment, K (kg water.m^{-2}. day^{-1}. Pa^{-1}) is the water vapour permeability of the packaging material, A_p (m^2) the surface area of packaging material, a_w (fraction) is the water activity of powder at T_a (˚C) and X_s (kg water.kg dry $solid^{-1}$) is the moisture content of the powder after a storage time of θ_S(day).

The time θ_S (days) required for the moisture content of the powder to increase from an initial value of X_i (kg water.kg dry $solid^{-1}$) to its critical value X_c (kg water.kg dry $solid^{-1}$) can be obtained by rearranging equation 3.20 as

$$\theta_s = \frac{W_d}{P^* K A_p} \int_{x_i}^{x_c} \frac{dX}{Rh_s - a_w} \qquad (3.21)$$

The value of the critical moisture content was taken as the moisture content at which lump formation and / or maximum browning was observed in the powder. The value of the integral of the equation above was estimated numerically by using the GAB equation.

The GAB model (Equation (2.3)) can be expressed in terms of a relationship between moisture content, X and the water activity of the sample as given in equation 3.22

$$X = \frac{a_w}{(\alpha a_w^{\ 2} + \beta a_w + \gamma)} \qquad (3.22)$$

Where,

$$\alpha = \frac{K}{M_o}\left(\frac{1}{C-1}\right) \qquad (3.23)$$

$$\beta = \frac{1}{M_o}\left(1-\frac{2}{C}\right) \qquad (3.24)$$

$$\gamma = \frac{1}{M_o CK} \qquad (3.25)$$

Equation (3.21) was solved by first differentiating equation (3.22) and substituting dX in terms of da_w in equation (3.21). Equation (3.21) was then integrated within the limits of initial and critical water activity to predict the shelf life of the product θ_S. The critical water activity of the sample was the value that corresponded to the critical moisture content based on browning / cakiness and was computed using the GAB model.

3.9.2 Lipid oxidation and shelf life prediction

Shelf life of cheese - *puri* mix is limited by lipid oxidation, which causes rancidity in the product, making the product unacceptable. The limiting value or the maximum value of peroxide for acceptance of the product beyond which the product become rancid for cheese – *puri* mix was determined by subjective evaluation of the flavour of the samples during accelerated storage (section 3.4).

In order to predict the shelf life of cheese - *puri* mix stored the volume of oxygen absorbed by the powder (cm^3.kg dry $powder^{-1}$) at the "maximum peroxide value" is also required. This volume of oxygen, absorbed by the powder when the peroxide value reaches its upper limit from acceptability was designated as $V_{O_{2max}}$. This was computed by experimentally establishing a relationship between the volume of oxygen absorbed by the powder and its corresponding peroxide value (equation 3.17).

Oxygen concentration inside the package corresponding to the calculated amount ($V_{O_{2max}}$) of oxygen absorbed was considered as the "final oxygen concentration" responsible for limiting the shelf life of the stored powder. This "final oxygen concentration", Y_f (volume fraction) was calculated using equation

$$Y_f = Y_a - \left(\frac{V_{O_{2\max}} W_d}{V_e} \right) \qquad (3.26)$$

Where, $V_{O_{2max}}$ (cm^3.kg dry $powder^{-1}$) is the maximum volume of oxygen absorbed, Y_f and Y_a (volume fraction) are the final and initial concentrations of oxygen present inside the package respectively V_e(cm^3) is the headspace volume inside the package and W_d (kg) is the dry weight of the powder stored inside the package.

The rate of change of oxygen concentration $dY/d\theta_s$ inside the package headspace was calculated as the difference between the rate of oxygen transfer into the package and the rate of oxygen absorption by the powder. This is expressed in the form of following equation

$$A_p K_o \left(Y_a - Y \right) - W_d R_y = V_e \left(\frac{dY}{d\theta_S} \right) \qquad (3.27)$$

Rearranging equation (3.26) we get,

$$\int_{X_i}^{X_C} \frac{dY}{d\theta_S} = -\left(\frac{W_d}{V_e} \right) R_y + \left(\frac{A_p K_o}{V_e} \right) \left(Y_a - Y_f \right) \qquad (3.28)$$

Where, W_d (kg) is the dry weight of the powder stored inside the package; $dY/d\theta_S$ is the rate of change of O_2 concentration Y within the package with the time of storage θ_S (day); V_e (cm^3) is the volume of headspace inside the package after placing the sample; A_p (m^2) is the surface area of the packaging material, K_O [$cm^3.day^{-1}.m^2$] is the oxygen permeability of the packaging material and R_y (cm^3oxygen. Kg of dry powder $^{-1}$ day^{-1}) is the rate of oxygen absorption by the powder.

Substituting the expression of R_y from equation 3.15 and simplifying the equation 3.28, the shelf life, θ_S can be expressed as

$$\theta_s = \int_{Y_a}^{Y_f} \frac{dY}{aa - bbY - ccY^2} \qquad (3.29)$$

Where, Y_f and Y_a (volume fraction) are the final and initial concentrations of oxygen present inside the package respectively and aa, bb, and cc are the constants whose values are given by,

$$aa = \frac{1}{V_e}(A_p K_o Y_a - W_d a_{01}) \qquad (3.30)$$

$$bb = \frac{1}{V_e}(A_p K_{oa} - W_d a_{02}) \qquad (3.31)$$

$$cc = \frac{W_d}{V_e} a_{03} \qquad (3.32)$$

Where α_{01}, α_{02} and α_{03} are regression constants of equation 3.15

Shelf life of the powder θ_S (days) was predicted by solving equation (3.29), integrated between the initial and final (critical) concentrations of oxygen present inside the package.

3.10 Validation of the developed models

The models developed to predict the shelf life of cheese – *puri* mix was validated by actual shelf life studies at 38˚C and 95 ± 2% RH. 200 g of cheese – *puri* mix was packed in LDPE (18 cm x 15.5 cm) pouches and placed in the regulated environment. Samples were drawn at frequent intervals and subjected to sensory evaluation by a trained panel and the quality of the product in terms of its colour and flavour was judged using a 9 - point hedonic scale.

Chapter- 4

Results and Discussion

Chapter-4

Results and discussion

This chapter comprises of the results obtained from the various experiments followed to fulfil the objectives undertaken to predict the shelf life cheese - *puri* mix. This chapter also covers the storage studies and sensory evaluation of the cheese - *puri* mix.

4.1 Proximate composition of cheese – *puri* mix

All samples of cheese - *puri* mix used for this study was prepared as per the procedure outlined in Fig 3.1. The composition of the mix was analysed using standard procedures for moisture content, fat, lactose, protein (section 3.3). The results for the proximate composition analysis are presented in Fig. 4.1.

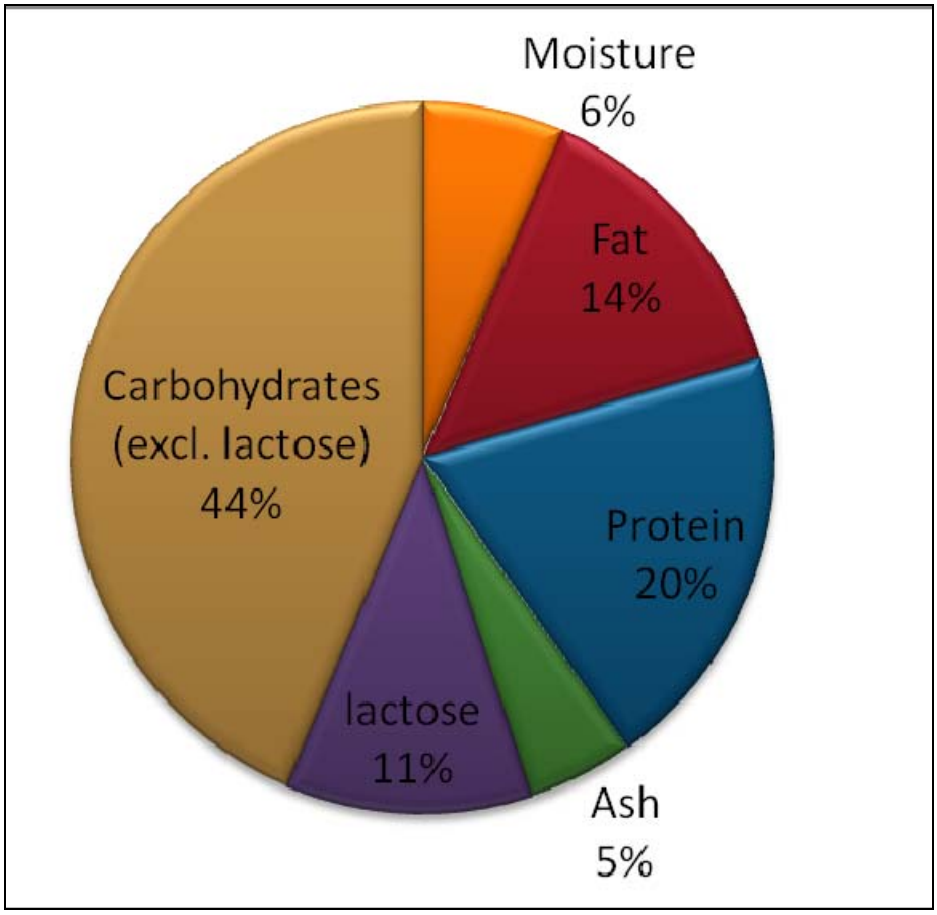

Fig. 4.1. Proximate composition of cheese *puri* mix

4.2 Sorption studies on cheese puri mix

4.2.1 Moisture sorption isotherms

Sorption studies on cheese - *puri* mix were carried out at temperatures of 25, 35, and 45˚C as per procedure described in section 3.4.1. The experimental data for equilibrium moisture content and water activity at different experimental conditions are presented in Appendix -2. Sorption isotherms were developed by using these experimental data. Fig.4.2 depicts the developed moisture sorption isotherms.

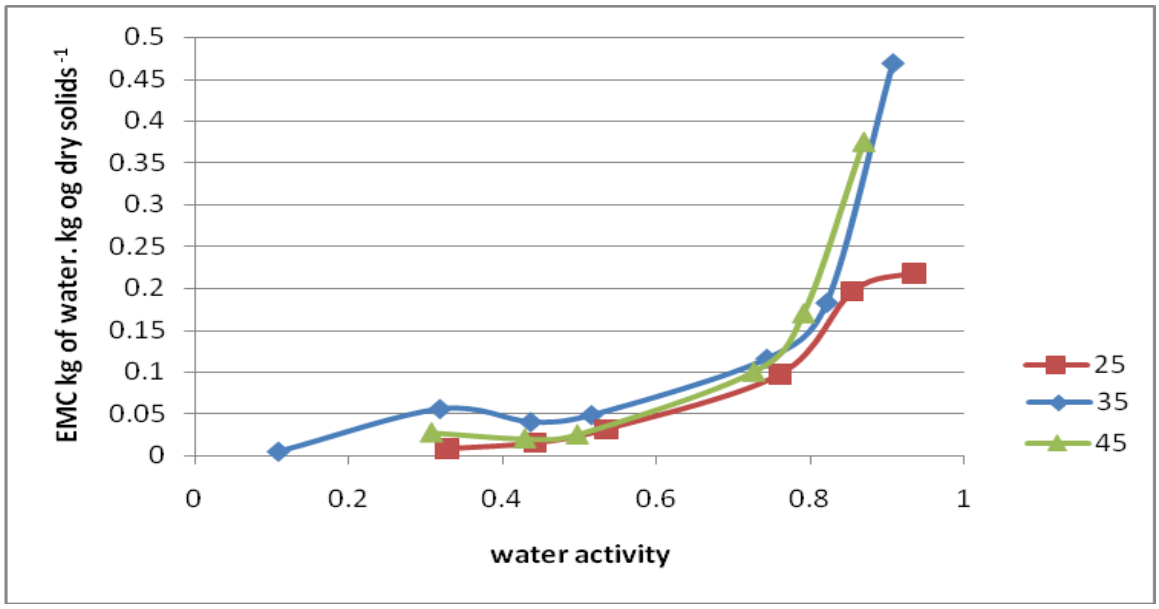

Fig. 4.2. Effect of temperature on moisture sorption isotherm of cheese puri mix

It was observed that an increase in temperature resulted in a slight decrease in equilibrium moisture content. This could be accounted to the fact that at increased temperatures, water molecules are activated to higher energy levels that allow them to break away from their sorption sites, thus decreasing the equilibrium moisture content (Palipane and Driscoll, 1992). However, the effect of temperature on equilibrium moisture contents of cheese - *puri* mix was found insignificant from the analysis of variance (ANOVA) of experimental data ($p<0.05$).

4.2.2 Modelling of sorption isotherm

GAB model (Table 2.2, equation 2.3) was fitted to the experimental moisture sorption isotherm data. Model constants, relative deviation percent, and average residuals for fitted model have been presented in the Table 4.1.

Table 4.1 GAB model constants

Model	Model parameter			R^2	E(%)
	C	K	M_o(%db)		
At 25˚C					
GAB	2.045	0.985	2.10	0.991	8.932
At 35˚C					
GAB	2.501	0.998	2.81	0.949	7.699
At 45˚C					
GAB	2.488	0.958	3.32	0.991	8.550

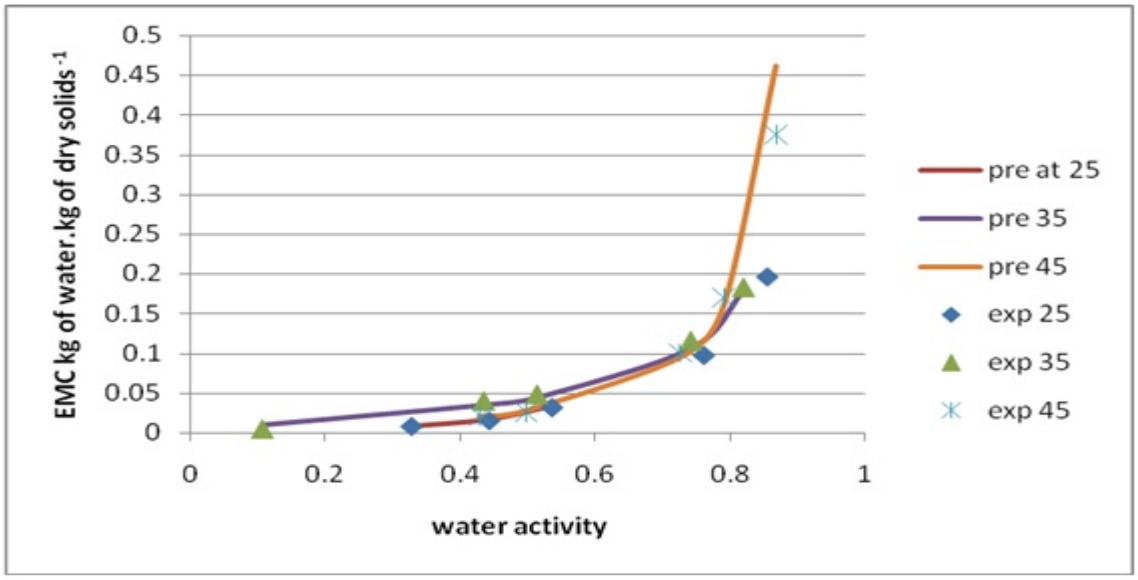

Fig. 4.3. Experimental (exp) and predicted (pre) relationship between water activity and equilibrium moisture content for cheese - *puri* mix (GAB model) at different temperatures

The experimental and predicted (GAB model) values of water activity and equilibrium moisture content for cheese - *puri* mix at different temperatures are presented in Fig 4.3. The relative deviation percents for the fitted model (Table 4.1.) computed for the different temperatures were lower than or close to 10%, indicating a very good fit of the model. This describes the adequacy of prediction of equilibrium moisture content values by the model.

4.2.3 Monolayer moisture content

Monolayer moisture content of a sample corresponds to the amount of moisture absorbed in a single layer to the binding sites of food material. Monolayer moisture content as obtained for cheese – *puri* mix from the GAB model was 0.0224±0.003 kg water.kg dry solids^{-1}. The effect of temperature on GAB monolayer moisture content values was not pronounced. The values of K of GAB model for the range of temperatures studied were closer to unity.

4.3. Water vapour transmission rate of packaging material

Low density poly ethylene (LDPE) pouch was selected as a packaging material for storage of cheese - *puri* mix. The measurement of water vapour transmission rate of this material was done by following the method described under the section 3.8. The gain in weight by calcium chloride placed in an aluminium cup sealed with the packaging material was monitored in an environment maintained at temperature of 38 ± 2˚C and 90 ± 1% RH. The average gain in weight was found to be linear with time (R^2 = 0.989) and is depicted in Fig 4.4.

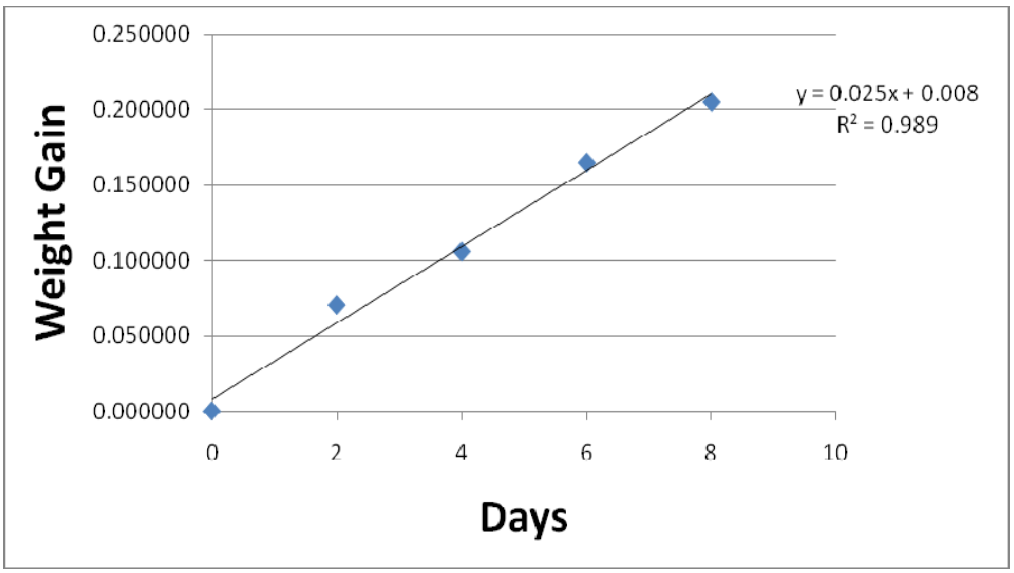

Fig. 4.4. Weight gain by the calcium chloride sealed in an aluminium cup with the packaging material

The slope of the best-fit linear plot (Fig. 4.4) was calculated as 0.025 g/day for LDPE. This value of slope was utilized in the equation (3.18) to compute the water transmission rate of packaging material. The surface area of the pouch (A_p) was 50 cm^2 and the saturation vapour pressure (P^*) of water at 38˚C was calculated to be 6539.03 Pa. Utilising these values in the equation (3.18), water transmission rate of the packaging material was found to be 5 g m^{-2} day^{-1}. This value is similar to WVTR reported for LDPE in literature (Wittuhm et al., 2005)

4.4 Oxygen permeability of the packaging material

For the measurement of oxygen permeability of LDPE, experiments were conducted according to the procedure detailed in section 3.9. The change in volume of the permeation was measured as a function of time as the displacement of a short column of coloured liquid in a glass capillary. The change in the volume was found to be 4.2 cm^3 for 4 seconds under constant pressure differential of 20 psi. Substituting this value in the equation 3.19, the oxygen permeability K_0 of the packaging material was calculated as 1600 $cm^3m^{-2}day^{-1}$. The obtained value was comparable to the oxygen permeability of 2000 $cm^3m^{-2}day^{-1}$ reported for LDPE (Wittuhm et al., 2005)

4.5 Shelf life prediction of cheese - *puri* mix

Mathematical models for predicting the shelf life of cheese – *puri* mix were developed based on the onset of non – enzymatic browning (NEB) and cakiness due to moisture absorption by the mix. The onset of oxidative rancidity was considered the deteriorative change in the product for predicting its shelf life based on absorption of oxygen.

4.5.1 Establishment of critical moisture levels

The theoretical basis for the model development based on moisture absorption by the packed cheese – *puri* mix sample is described under section 3.10. In order to establish the critical levels of moisture content for acceptability of cheese - *puri* mix, the powder with an average initial moisture content of 0.0638 kg of water per kg of dry solids was subjected to accelerated storage studies (45 ± 1˚C, 95 ± 2% RH) and the samples were subjectively evaluated at periodic intervals. The critical moisture content for cheese - *puri* mix was determined separately for the two deteriorative changes due to moisture absorption, namely, non - enzymatic browning in the powder and cakiness.

4.5.1.1 Critical moisture based on non - enzymatic browning

In addition to the subjective evaluation to identify the exact point of rejection of the sample, non - enzymatic browning in the powder was also objectively measured using the following methods.

4.5.1.1.1 Measurement of Browning Index

The browning index of the powder was measured by using an enzymatic digestion method (Palombo et al 1984) following method described in section 3.5.1.1. In order to establish a relationship between browning index and moisture gain by the sample, the corresponding moisture content of the sample was determined using the gravimetric method (section 3.3.1). The moisture content, browning index and the sensory scores for colour of powder with storage time are tabulated in the Table 4.2. The values for browning index obtained for the

product were comparable to the range of browning index reported during storage of dried cheddar cheese powder (Kilic et al., 1997)

Table 4.2 Moisture content and corresponding browning index values for cheese *puri* mix

Moisture content (%db)	Moisture content (kg of water per kg of dry solids)	Browning index (OD/g of dry solids)	Sensory Score for colour
6.7294	0.0672	0.56	8.0
13.8995	0.1389	1.113	7.8
15.1455	0.1514	1.192	7.5
16.7	0.1670	1.597	6.8
21.1535	0.2115	2.994	5.2
23.34	0.2334	3.225	4.8

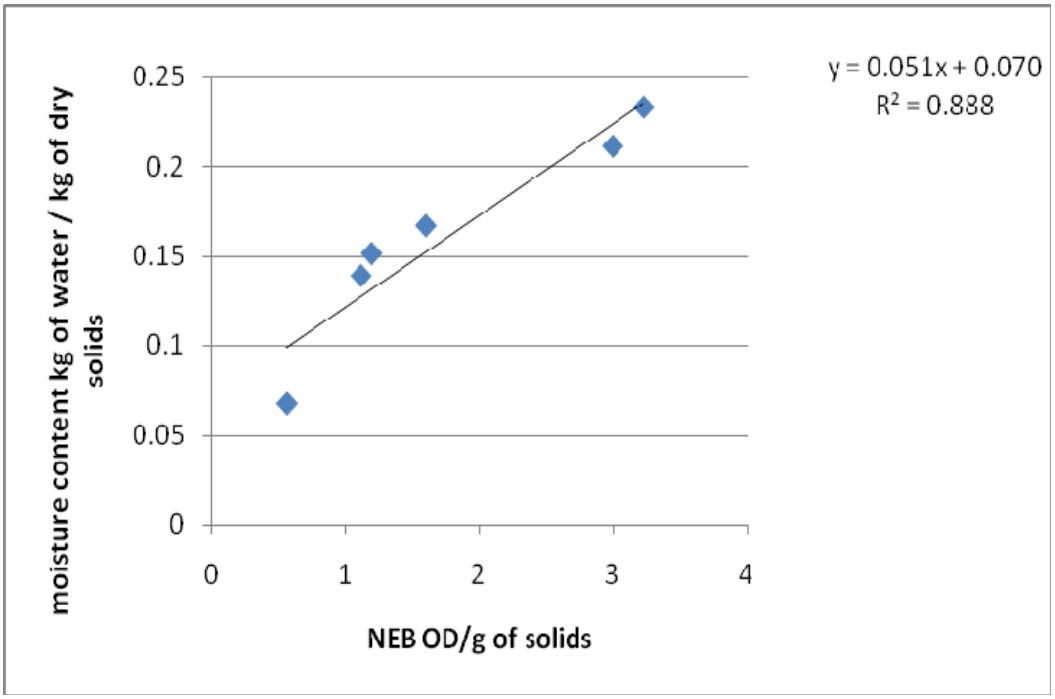

Fig. 4.5. Effect of moisture content on non-enzymatic browning (NEB)

Sample after browning **Fresh sample**

Fig. 4.6 Sample after browning and fresh sample

The moisture gain by cheese – *puri* mix corresponded linearly with the NEB of the sample (Fig. 4.5., equation 4.1)

$$M.C. = 0.051 (NEB) + 0.070 \quad (4.1)$$

Where, M.C. = moisture content of sample, fraction (d.b.)

NEB = Browning Index of the sample, OD / g of solids

Critical moisture content for non-enzymatic browning was the moisture content at which product became unacceptable due to maximum browning. Critical NEB was established by sensory evaluation (Table 4.2) and the corresponding browning index of the sample was determined 2.994 OD/g of total solids. The corresponding critical moisture content was determined from the linear equation (4.1) and it was calculated as 0.2226 kg of water.kg of dry solids^{-1} (22.26 % db).

4.5.1.1.2 Total Colour Difference of the sample

The extent of browning in the sample was also quantified by measuring the colour of the sample by digital imaging technique (section 3.5.1.2). The computed values of ΔE (equation 3.11) and the corresponding moisture content of stored powder against the period under accelerated storage are tabulated in the Table 4.3.

Table 4.3. Moisture content and corresponding ΔE values for cheese - *puri* mix

Days	L	L*	a	a*	b	b*	ΔE	M.C (%db)
0	221.58	86.89	132.28	4.498	160.25	30.823	0	6.729
11	216.88	85.05	133.55	5.694	161.02	31.548	2.310	10.325
24	212.78	83.444	131.1	3.388	160	30.584	3.629	13.391
30	211.48	82.93	135.95	7.952	162.47	32.912	5.652	13.830
37	209.1	82.00	136.71	8.668	163.65	34.023	7.179	17.247
42	212.06	83.160	137.6	9.505	163.78	34.145	7.072	15.714
52	208.22	81.654	138.44	10.297	165.36	35.632	9.173	17.841
56	208.83	81.894	138.22	10.089	164.46	34.785	8.480	16.686
56	205.47	80.576	139.82	11.595	166.5	36.705	11.172	21.720
68	202.07	79.243	135.43	7.4635	168.05	38.164	11.007	21.813

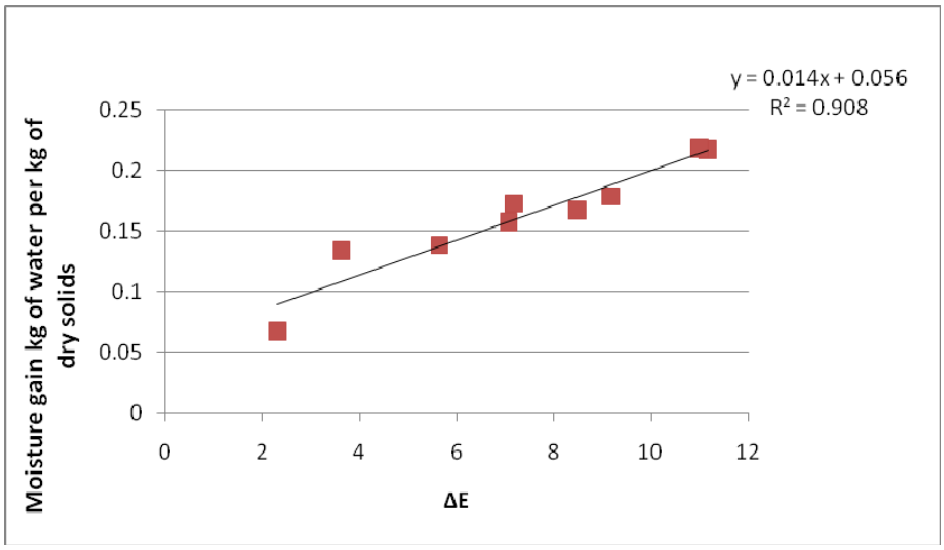

Fig. 4.7. Effect of moisture content on total colour change (ΔE) values and sensory score of cheese- *puri* mix

The moisture content of the sample was found to vary linearly with its ΔE values (Fig. 4.7.) and fitted the equation (4.2).

$$M.C. = 0.0014\ (\Delta E) + 0.056 \qquad (4.2)$$

Where, M.C. = moisture content of sample, fraction (d.b.)

ΔE = total colour difference of the sample

The sample was found to be rejected due to browning during sensory evaluation at a ΔE value above 11. The corresponding critical moisture content was computed using equation 4.2. and found to be 0.2124 kg of water. kg of dry solids^{-1} (21.24% db).

4.5.1.2 Critical moisture based on cakiness

Cakiness of the product was determined based on the sticky point temperature (T_S) of the product, determined as the point at which a sharp increase in torque was observed during shearing of the product. Such sharp increase in the torque is reportedly typical for powdery materials (Kudra, 2003), while for pasty materials a gradual increase in torque is experienced yielding a sticky region instead of a sticky point.

The moisture content at which stickiness was first observed in the product was considered as the critical moisture content that limits the shelf life of the product due to cakiness. The sticky point temperature for a product was determined at different moisture content and the variation in the sticky point temperature of cheese – *puri* mix at different moisture is given in the Table 4.4. The sticky point temperature of the product was determined to be 67.25 ^{o}C at a moisture content of 7.5 % (db). This value was comparable to the sticky point temperature of >70 ˚C reported for tomato soup powder at the moisture level of 5 % (db) (Jaya and Das, 2009)

Table 4.4 Sticky point temperature and the corresponding moisture content of cheese - *puri* mix

Moisture content(kg of water/ kg of dry solids)	Sticky point temperature(˚C)
0.07489	67.25
0.1098	63.4
0.1438	57.15
0.1640	51.55
0.1955	47.35
0.2146	38.80
0.303	28.00

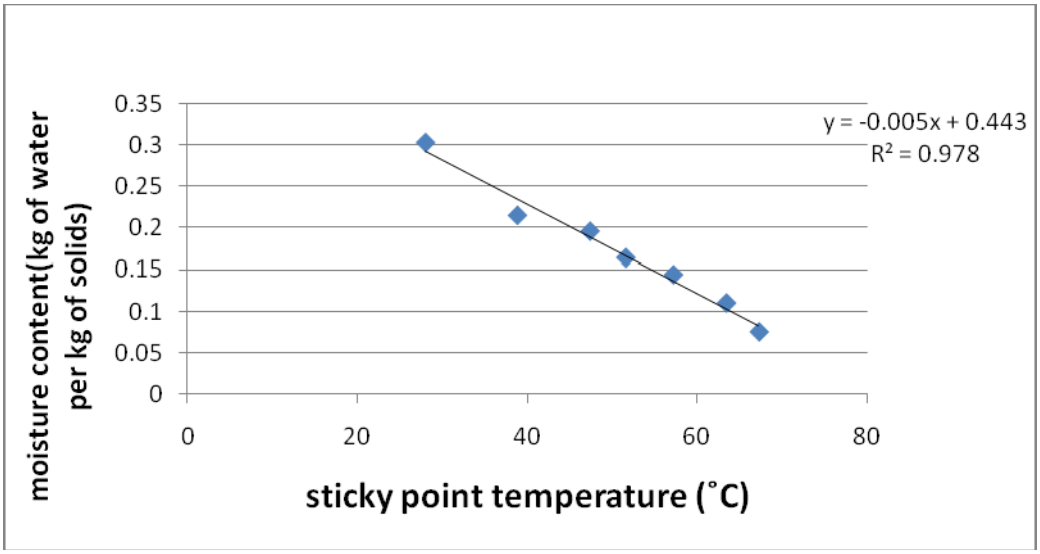

Fig. 4.8. Effect of moisture content on sticky point temperature of cheese - *puri* mix

The sticky point temperature of cheese – *puri* mix sample was found to linearly vary with the moisture content of the sample (Fig. 4.8) and was found to follow equation (4.3)

$$M.C. = -0.005\ T_S + 0.443 \qquad (4.3)$$

Where, M.C. = moisture content of sample, fraction (d.b.)

T_S = Sticky point temperature, ^oC

The critical moisture content at which cakiness sets in the powder was computed from the above linear equation (4.3). At a storage temperature of 38˚C, it was found to be 25.3 (%db) or 0.253 kg of water. Kg of dry solids^{-1}.

4.5.2 Shelf life based on absorption of moisture

The shelf life of the cheese - *puri* mix, based on absorption of moisture was predicted using Equation (3.21). It was determined as the time required for the powder to reach the critical moisture content. Amongst cakiness and non - enzymatic browning, the critical moisture content (X_c) for NEB was found to be 21.75 % d.b. (average of critical values from browning index and ΔE) while for cakiness it was found to be 25.30 % d.b. Since X_c for NEB was less than the X_c for cakiness, the critical moisture content X_c for cheese - *puri* mix based on deterioration due to moisture absorption was fixed as the moisture content at which the maximum browning in the powder was observed and it was taken as 0.2175 kg of water. kg of dry solids^{-1}.

Equation 3.21 was solved by substituting moisture content, X, as a function of water activity, a_w, based on the GAB model (equation 3.22). It was assumed that the variation in the GAB model constants between 35 and 38 ^oC would be negligible. The value of the integral of equation (3.21) was now estimated numerically between the limits a_{wi} = 0.26 and a_{wc} =0.86, which corresponded to the initial water activity of the sample and its water activity computed at the critical moisture content of 21.75 % (db), respectively.

Table 4.5 Values of parameters used to solve equation 3.21

Parameter	Unit	Value input
Water vapour permeability, k	$Kg.m^{-2}.day^{-1}Pa^{-1}$	WVTR / P*
Water vapour Transmission Rate, WVTR	$Kg.m^{-2}.day^{-1}$	5x1000
Saturated water vapour pressure at 38˚C, P^*	Pa	6539.03
Package surface area, A_p	m^2	0.0279*2 = 0.0558
Dry weight of sample, W_d	kg	0.188

The critical limit for the water activity was slightly higher than the range of a_w (0.60 - 0.80) reported for non-enzymatic browning for foods (Labuza and Saltmarch, 1981), probably due to the influence of spices like chilli powder on the acceptable colour of the product during subjective evaluation. By substituting the values of P^*, W_s, A_p and K listed in Table 4.5 in equation (3.21), the shelf life of the cheese - *puri* mix was predicted to be 270 days.

4.5.3 Establishment of critical limits for lipid oxidation

Shelf life prediction of packaged cheese - *puri* mix necessitates modelling of oxygen absorption by the powder. Experiments were conducted in sealed plastic bottles (made leak- proof using silicone tape) to find out the relationship between volume of O_2 absorbed and the peroxide value developed in the powder (equation 3.17). Experiments were carried out as per the plan described in the section 3.6 – 3.7. Experimental data on the variation of oxygen concentration and peroxide value of the cheese - *puri* mix (stored in the plastic bottles) with time of storage is presented in the Table 4.6. From Table 4.6, it is observed that as the time of storage increases, concentration of oxygen in the headspace of the container decreases whereas the peroxide value increases. Volume of oxygen absorption and peroxide value variation were modelled using the experimental data.

Table 4.6. Variation of oxygen concentration and peroxide value of cheese puri mix stored in sealed plastic bottles.

Time of storage (days)	O_2 concentration inside the bottle headspace,Y (volume fraction)	Peroxide value of powder (m.Eq.of O_2.kg fat^{-1})	Rate of O_2 absorption, R_y ($cm^3.day^{-1}$. kg dry $powder^{-1}$)	Volume of O_2 absorbed, Vo_2 ($cm^3.day^{-1}$.kg dry $powder^{-1}$)
0	0.2145	1	-	0
7	0.2125	1	3.1914	22.3404
14	0.211	1.2	3.1914	44.6808
21	0.2105	1.4	2.4822	52.1276
28	0.21	2	2	59.5744
35	0.21	2.4	1.7021	59.5744
42	0.2095	2.8	1.5957	67.4680
49	0.209	3.2	1.5197	74.4680
56	0.2075	4	1.7287	96.8085

4.5.3.1 Rate of oxygen absorption

Volume of headspace inside the plastic bottles after placing the cheese - *puri* mix was calculated by subtracting the powder volume from the total volume of air (40 cm^3) present initially and adding the volume of air present within the void space of the powder. The powder volume and void volume were calculated to be 10 and 5 cm^3 respectively. Porosity of the cheese - *puri* mix was assumed as 50%. The total volume V_e of air present inside the headspace was estimated to be 35 cm^3 and the dry weight of the powder W_d was 0.0047 kg.

Using the values of V, W_d, the time of storage (θ_i) and the corresponding oxygen concentration (Y_i) (Table 4.5) in equation (3.13), values of rate of oxygen absorbed R_y ($cm^3.day^{-1}$.kg dry $powder^{-1}$) by the cheese - *puri* mix was calculated. The calculated R_y values are reported in Table (4.5). The average oxygen concentration Y (fraction) present in the headspace of the plastic bottles at different time intervals were estimated by using equation (3.14). Second order non - linear regression equation of type (equation 3.15) was developed using the

values of R_y and Y. The developed regression equation is presented as equation (4.4).

$$R_y = 269.0 - 3059*Y + 8510*Y^2 \qquad (4.4)$$

Where, R_y = rate of O_2 consumption by the powder, $cm^3.day^{-1}.kg\ dry\ powder^{-1}$

Y = concentration of O_2 inside the bottle, volume fraction.

4.5.3.2 Modelling of peroxide value and the amount of oxygen absorbed

Peroxide values (PV) of the cheese - *puri* mix measured at different times of storage inside the bottle are given in Table (4.5). Using the values of V_e (35 cm^3), W_d (0.0047 kg) and the O_2 concentration Y (Table 4.5), volume of O_2 absorbed by the powder (V_{O_2}) after different time of intervals were calculated using equation (3.16). Experimental data of peroxide value and Vo_2 were fitted to a liner model, equation (4.5), which was found to be the best fit.

$$PV = 0.033V_{O_2} + 0.329 \qquad (4.5)$$

Fig. 4.9 depicts the graphical representation of equation (4.5) with the experimental data Equations (4.4) and (4.5) were used for the shelf life prediction of cheese - *puri* mix.

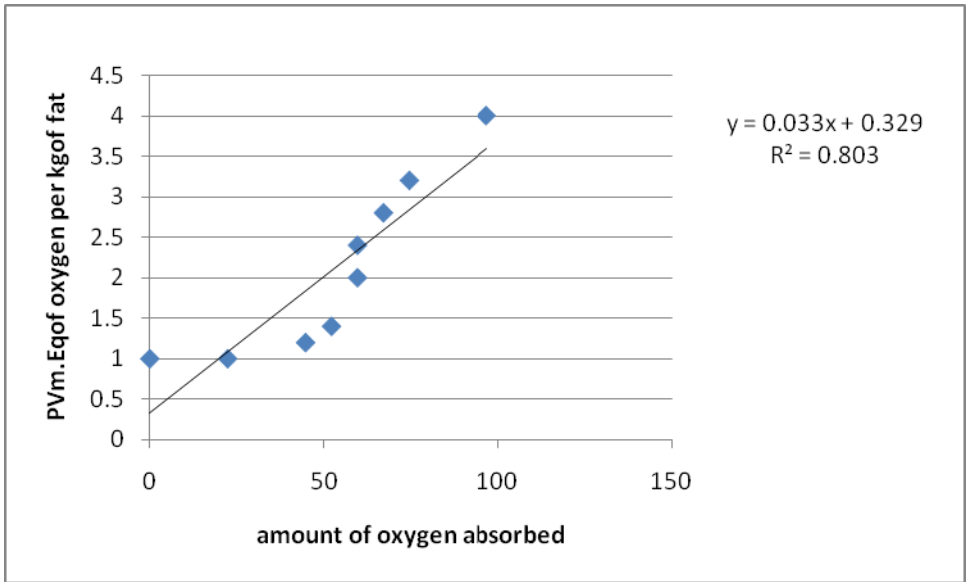

Fig. 4.9. Relationship between peroxide value and volume of O_2 absorbed by the cheese puri mix

Table 4.7. sensory scores of cheese *puri* mix for critical rancidity

Storage period, days	Sensory scores for flavour	Peroxide values, mEq kg fat^{-1}
0	8	1.0
11	7.9	1.0
24	7.8	1.4
30	7.5	1.4
37	7.5	2.0
42	6.5	2.8
52	6.2	3.2
60	5.5	3.4

4.5.3.3 Critical limit for oxygen content

Sensory evaluation of the samples subjected to accelerated storage (section 3.5) established that a peroxide value of 3.4 mEq.of O_2.kg of fat^{-1} was considered the maximum limit of peroxide value for cheese - *puri* mix to be considered rancid free (Table 4.7.). Volume of of O_2 absorbed corresponding to the peroxide value (Vo_{2max}) of 3.4mEq.of O_2.kg of fat^{-1} was estimated from the equation 4.5. This value of $V_{O_{2max}}$ was calculated to be 93.060 cm^3.kg of dry $powder^{-1}$.

The headspace volume V_e inside the LDPE pouches (18 cm x 15.5 cm) after placing a sample was calculated to be 247 cm^3. Initial concentration of O_2, Y_a,(volume fraction) inside the pouches was 0.214. Substituting the values of W_d, V_e, Y_a and Vo_{2max} into equation (3.26), Y_f (final concentration of O_2 inside the package corresponding to (Vo_{2max}) was predicted. The value of Y_f (volume fraction) thus calculated was 0.143 (14.3%).

4.5.4 Prediction of shelf life based on oxidative rancidity

The time required to attain the headspace O_2 concentration of Y_f = 0.143 was considered to be the predicted shelf life of the packaged cheese - *puri* mix. Equation (3.29) was used to predict the shelf life of cheese puri mix stored in

LDPE pouches. The constants aa, bb, and cc of equation (3.29) were calculated using the relationships given in equations (3.30 – 3.32) using the values of various parameters as listed in Table 4.8.

Table 4.8. Values of parameters used to solve equations (3.30 – 3.32)

Parameter	Unit	Value input
Oxygen permeability, k_O	$cm^3.m^{-2}.day^{-1}$	1600
Initial oxygen concentration, Y_i	fraction	0.214
Package surface area, A_p	m^2	0.0279*2 = 0.0558
Dry weight of sample, W_d	kg	0.188
a_{01}	Constant*	269
a_{02}	Constant*	-3059
a_{03}	Constant*	8510

*obtained from equation (4.4)

The calculated values of aa, bb, and cc in equation (3. 29) were presented in Table 4.9.

Table 4.9. Values obtained for constants from equation (3.30 – 3.32)

Parameter in equation (3.29)	Computed value (from equation 3.30 - 3.32)
aa	- 0.1173
bb	-1.96
cc	6.4635

Integration of equation (3.29) was carried out numerically between the limits of initial oxygen concentration, Y_a = 0.214 and final (critical) headspace oxygen concentration, Y_f = 0.143, by using these calculated values of aa, bb, and cc. The shelf life cheese - *puri* mix predicted from the solution of equation (3.29) was estimated to be 180 days.

4.6 Shelf life studies on cheese – *puri* mix

To validate the developed models for predicting shelf life of cheese – *puri* mix, actual shelf life of the product was determined as described in section 3. 11. The sensory panel were presented samples of cheese – *puri* mix, packed in LDPE pouches and stored at 38˚C and 95 ± 2% RH, at frequent intervals. The samples were judged for its colour and flavour on a 9 – point hedonic scale and the average scores are presented in Fig. 4.10.

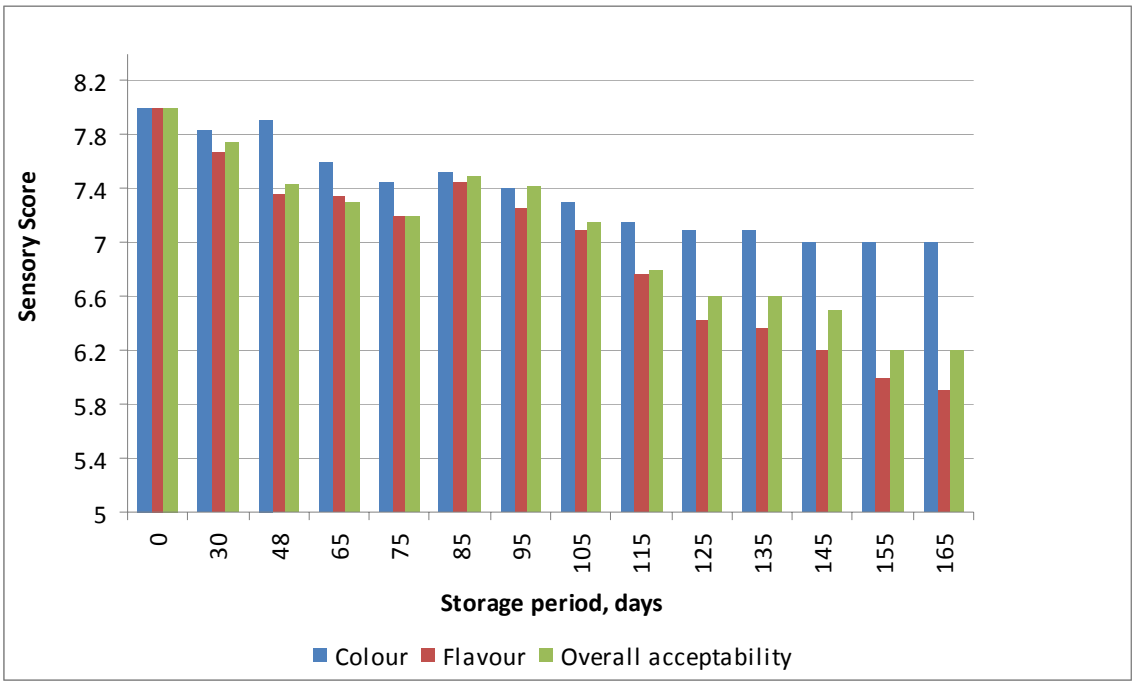

Fig. 4.10. Sensory scores for Cheese – *puri* mix during actual shelf life studies

It was observed that the sensory scores for colour of the stored product remained fairly steady for about 48 days of storage and thereafter showed a marginal decline from scores of 7.9 -8 to about 7.5 at 65 days of storage. After 105 days of storage time, sensory scores for the colour of the product declined further, scoring about 7.0 towards the latter period of storage. Comparatively, the flavour scores of the product showed a more rapid decline, the product scored an average of 7.3 at the 48th day of storage.

The developed models predicted a shelf life of 270 days based on moisture absorption, while the shelf life based on oxidative rancidity was predicted to be 180 days. This indicated that the sample would spoil earlier due to rancidity development compared to browning. This observation was validated during sensory evaluation of the stored samples; the stored samples were found to get better score for colour compared to its flavour score. After 105 days of storage, the product progressively scored poorly for flavour; scoring < 6.0 after 165 of storage days and the panel commented on the onset of a rancid odour in the sample. Therefore, the actual shelf life of a cheese - *puri* mix was deemed to be 165 days. This showed a 90 % agreement with the predicted value of 180 days, indicating that the developed models could be successfully applied to predict the shelf life of cheese – *puri* mix.

Chapter- 5

Summary and Conclusion

Chapter – 5
Summary and Conclusions

An attempt was made to develop mathematical models to predict the shelf life of cheese – *puri* mix. Two approaches were considered to develop the model; based on the onset of deterioration due to moisture absorption by the product and due to the development of lipid oxidation.

Moisture adsorption behavior of cheese – *puri* mix was studied by sorption isotherms determined at 25, 35 and 45^{O}C using the gravimetric method. Samples were equilibrated in desiccators containing saturated salt solutions of known water activity (0.11 – 0.97). Water activity and equilibrium moisture content data obtained from the sorption studies were fitted to the GAB model and the GAB model parameters were estimated using direct nonlinear regression analysis.

Deterioration of the product due to absorption of moisture was anticipated to be manifested as non – enzymatic browning (NEB) and cakiness. NEB in the product was quantified in terms of its browning index and total colour difference. Browning index was determined using enzyme digestion of the sample and expressed as the difference in the optical density of the sample at 320 and 550 nm. The total colour difference in the sample was measured using image analysis of the sample. Development of cakiness in the sample was related to its sticky point temperature, which was determined at the temperature at which a sudden change in torque was required to shear the product.

Mathematical relationships were developed to predict the peroxide value of the product in terms of the volume of oxygen absorbed by the mix and to predict the rate of oxygen absorption by the product as a function of its headspace oxygen concentration. The maximum acceptable level of deterioration in the product was subjectively evaluated at 45 ˚C and 95 % RH and the corresponding critical levels of moisture and oxygen absorption in the sample were established.

Low density polyethylene (LDPE) (350 gauge) was selected as the packaging material for the product. The packaging material was characterized for its water vapour transmission rate (WVTR) and oxygen permeability as per ASTM E96 and ASTM D1434, respectively.

Independent models based on moisture absorption and lipid oxidation were developed and validated for the prediction of shelf life of cheese – *puri* mix (200g) packed in LDPE pouches (18 cm x 15.5 cm) at 38˚C and 95 ± 2% RH.

The pertinent results and conclusions drawn from this research work are summarized below.

i. Sorption isotherms for cheese – *puri* mix were determined at 25, 35 and 45 ^{O}C and there was good agreement between the experimental sorption data and calculated values using GAB's equation.

ii. The acceptable limits for non - enzymatic browning based on browning index of cheese – *puri* mix was sensorily determined as 2.994 OD per g of dry solids and < 11 for total colour difference. The corresponding critical level for moisture in the product for development of NEB was computed to be 21.75 % (db).

iii. Critical moisture content of the product for development of cakiness due to moisture absorption was determined by sticky point evaluation as 25.3 % (db).

iv. Rate of oxygen absorption by the product was found to be a polynomial function of its headspace oxygen concentration.

v. The amount of oxygen absorbed by the product and its peroxide value (PV) were found to follow a linear relationship.

vi. Sensory evaluation of the product revealed that the maximum PV of 3.4 m.Eq per kg fat was acceptable and the corresponding amount of oxygen absorbed was found to be 93 cm^3 kg dry $solids^{-1}$.

vii. The WVTR of LDPE (350 gauge, thickness 87.5 μ) was determined as 5 g m^{-2} day^{-1}; while its oxygen permeability was found to be 1600 cm^3 m^{-2} day^{-1}.

viii. The shelf life of cheese - *puri* mix packed in LDPE pouches (200g) stored at 38˚C, 95 % RH was predicted as 270 days based on moisture absorption and 180 days based on lipid oxidation.

ix. Actual shelf life testing at 38˚C, 95 % RH revealed that the sample spoilt faster due to rancidity development. This was in accordance to the spoilage trend projected by the models, as the predicted shelf life based on lipid oxidation was less than that due to moisture absorption.

x. The actual shelf life obtained by subjective evaluation was found to 165 days (for flavour), which showed a 90% agreement with the predictive model developed.

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Appendices

APPENDIX I

Equations used for calculation water activity at different temperature for saturated salt solutions

Saturated salt solution	Equation used
LiCl	$\ln a_w = 500.95[1/T]-3.8$
$MgCl_2.6H_2O$	$\ln a_w = 303.35\ [1/T]-2.1$
K_2CO_3	$\ln a_w = 145[1/T]-1$
$Mg(NO_3)_2.\ 6H_2O$	$\ln a_w = 356.6\ [1/T]-1.8$
NaCl	$\ln a_w = 228.92\ [1/T]-1.0$
KCl	$\ln a_w = 367.58\ [1/T]\ -1.3$

APPENDIX II

Moisture sorption data for cheese - *puri* mix

At 25˚C		At 35˚C		At 45˚C	
a_w	**EMC (kg water.kg dry solid^{-1})**	**a_w**	**EMC (kg water.kg dry solid^{-1})**	**a_w**	**EMC (kg water.kg dry solid^{-1})**
0.328	0.0074	0.108	0.00522	0.308	0.0276
0.443	0.0146	0.318	0.05601	0.429	0.0204
0.536	0.0313	0.436	0.0404	0.497	0.0254
0.761	0.0972	0.515	0.04869	0.726	0.1003
0.855	0.1965	0.743	0.1159	0.791	0.1702
0.936	0.2187	0.821	0.1829	0.869	0.3753
		0.907	0.4681		

a_w- water activity fraction, EMC- Equilibrium moisture content

APPENDIX III

DAIRY TECHNOLOGY SECTION

NATIONAL DAIRY RESEARCH INSTITUTE, BANGALORE

SENSORY EVALUATION SHEET

(9 POINT HEDONIC SCALE)

Product : Cheese puri mix **Date:**

Name of the judge:

Characteristics	Samples					
	1	2	3	4	5	6
Colour and appearance						
Flavour						
Body and texture						
Overall acceptability						

Remarks:

9- Like extremely 8- Like very much 7-Like moderately 6- Like slightly 5- Neithe like nor dislike 4- Dislike slightly 3- Dislike moderately 2- Dislike very much 1- Dislike extremely