DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILM FOR PACKAGING OF MILK CAKE

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

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(Minor Subject: Dairy Engineering)

By

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(L-2016-D-05-M)

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CERTIFICATE – I

This is to certify that the thesis entitled, **"DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILM FOR PACKAGING OF MILK CAKE"** submitted for the degree of **M.Tech.**, in the subject of **Dairy Technology** (Minor Subject: **Dairy Engineering**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Navdeep Singh (L-2016-D-05-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

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ABSTRACT

Packaging is one of the main phenomenon or operation used for the preservation of edible products in order to increase the shelf life. Generally different materials such as plastics, polyethylene are used as packaging material for packaging of different food materials. These packaging materials are non-degradable, which cannot easily degrade and cause environment pollution. In recent years, India has a substantial growth in consumption of plastics and an increased production of plastic waste, where packaging is major plastic consuming sector with 42% of the total consumption, so these packaging materials needs to be replaced and it can be done with edible and biodegradable packaging. In the present study, edible packaging films were prepared using different sources such as starches (corn starch, wheat starch and rice starch) and protein (sesame protein isolate & soy protein isolate) along with different plasticizers such as glycerol, sorbitol and mannitol to increase the flexibility of the film. Drying conditions for film preparation was at 40^{o}C for 16-18 hours. The quantity of polysaccharides and plasticizer were optimized for making proper form of edible films. Different mechanical and physico-chemical properties of those films were analysed such as thickness, tensile strength, water vapour transmission rate, water solubility, moisture content, puncturing strength, color analyses, water activity and transmittance. By analyzing these properties, it was found that the corn starch (5%) and glycerol (2%) gave effective result compared to other carbohydrate or protein-based polymers. It had minimum thickness, better water solubility, high tensile and puncturing strength compared to other combinations tested. Storage study was conducted at two different temperatures i.e. Refrigeration temperature (4°C) and ambient temperature (25°C), for different storage parameters such as FFA, HMF, Peroxide value, Tyrosine Value, TBA value, Reducing sugars etc. All these values increased with the storage period and increased significantly either on third or sixth day of storage. Physico-chemical properties such as moisture, ash & pH values showed significant decrease in values with time. However, increase the value of titratable acidity, microbiological properties such as SPC, coliform & Yeast and mold was observed stating the post processing contamination or attributed to conditions such as aerial contamination. A life of 15 days at refrigeration and 6 days at ambient temperature was recorded for control and wrapped product both, respectively. Consumer acceptability studies stated extremely liked and liked very much by the consumers to a major extent revealing suitability of the product, if commercialized.

Keywords: Edible film, milk cake, packaging, shelf life, storage.

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Signature of Major Advisor Signature of the Student

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ABBREVIATIONS

AG	:	Agar
CA	:	Citric Acid
CAS	:	Cassava Starch
CEO	:	Cinnamon Essential Oil
CMC	:	Carboxy Methyl Cellulose
DMSO	:	Dimethyl Sulphoxide
DSC	:	Differential Scanning Calorimetry
EMC	:	Equilibrium Moisture Content
FFA	:	Free Fatty Acids
FS	:	Film Solubility
GLU	:	Glutaraldehyde
GLY	:	Glycerol
HMF	:	Hydroxy Methyl Furfural
HPMC	:	Hydroxy Propyl Methyl Cellulose
LMWC	:	Lower Molecular Weight Chitosan
LPOS	:	Lactoperoxidase System
MC	:	Moisture Content
NRS	:	Normal Rice Starch
OP	:	Oxygen Permeability
OPS	:	Oxidized Potato Starch
PEG	:	Polyethylene glycol
PUL	:	Phullulan
PV	:	Peroxide Value
RH	:	Relative Humidity
RVP	:	Relative Vapor Pressure
SEM	:	Structural Electron Microscope
SPC	:	Standard Plate Count
TBA	:	Thio Barbituric Acid
TEM	:	Transmission Electron Microscope
TEMPO	:	2, 2, 6, 6-tetramethylpiperidine-1-oxyl
TPS	:	Thermoplastic Starch
UTS	:	Ultimate Tensile Strength
WPC	:	Whey Protein Concentrate
WPI	:	Whey Protein Isolate
WRS	:	Waxy Rice Starch
WVP	:	Water Vapor Permeability
WVTR	:	Water Vapour Transmission Rate

CHAPTER – I

INTRODUCTION

India retained its number one ranking in milk profaduction with an expected output of 164 million tons in 2016-17 (Anon 2017). In India, about 46 per cent of the total milk produced is consumed as liquid milk and 47 per cent is converted into milk traditional products like Cottage Butter, Ghee, *Paneer, Khoa,* Curd, *Malai* and *Mithais*, the delectable world of Indian milk delicacies (Saxena 2007). The dairy industry of India has indeed witnessed significant progress during the last two decades and amongst various categories the indigenous dairy products are extremely popular in the country. Milk sweets and delicacies have been an integral part of social and cultural heritage of India. Several types of milk sweets are prepared and consumed in different parts of the country owing to their rich aroma and pleasant taste. In the present context, *Kalakand, Khurchan, Rabri*, *Burfi*, Milk cake, etc. represent a range of commercially important heat desiccated milk products. The place of these products in the dietary regime as well as socio-economic connotations remains significant through our cultural heritage. Amongst these, Milk cake is one of the popular sweets of Northern and central parts of India gaining popularity in the other parts of the country as well. The product is a rich source of energy, milk proteins, minerals and other growth promoting factors (De 1980).

Milk cake is characterized by well-defined grains having more pronounced caramel flavor than Kalakand which is prepared from milk with characteristic of danedar (granular) variety of Khoa (Mathur1991). Different researchers like Karwasra *et al* (2001), Madhava Rao *et al* (2003) and Landge *et al* (2009) have standardized the method for its preparation without standardizing the stage of addition of citric acid or sugar. However, during storage the product tends to pick up odors from surrounding atmosphere and lose its typical aroma and is also prone to oxidative rancidity. In this content, very little attention is paid to packaging and sanitary handling practices (Patil 2003). On the commercial scale, the very challenge faced by the manufacturers is low shelf life of the products (Misra2000; Aneja *et al* 2002; Patil 2005). Conventionally, paperboard is used for packaging of these sweetmeats. Adding to the limitations, this packaging material is discarded with very little being recycled, which results in disposal problem for paper and cardboards which causes environmental pollution, especially in case of plastic packages. Also, uncontrolled dumping of waste on

outskirt of town and cities has created overflowing landfills which cause serious environmental implication in term of ground water pollution and contribution to global warming (Gupta *et al* 1998). The most recently compiled waste generation statistics indicated that 245.7 million tons of MSW (Municipal Solid Waste) was generated in 2005, out of which approximately 168 million tons (68%) of MSW was discarded into the municipal waste stream of which 33.4 million tons (20%) was combusted prior to disposal (Marsh *et al* 2007) and 133.3 million tons was directly discarded in landfills. In recent years, India has a substantial growth in consumption of plastics and therefore an increased production of plastic waste, where packaging is major plastic consuming sector with 42% of the total consumption (Mutha *et al* 2006). Therefore, the interest of researchers and scientists has grown for edible film and coatings in recent years. The edible films also enhance the aesthetic properties if sweetener, coloring agents are added in the film, apart from giving various barrier characteristics.

The shelf life of a heat desiccated dairy products is generally dependent on the post-production conditions because such products are almost sterile when produced and hence such products get spoiled due to post-production contamination. The solution to this problem is development of films which may be edible or non-edible for packaging of heat desiccated milk products. These films cannot totally replace synthetic packaging but can limit moisture, aroma and lipid migration from food. The present demand of consumers is increasing day by day for natural and organic foods. New Technologies in natural or organic foods offer number of products with improvements in quality, freshness as well as food safety, thus it has forced companies and researchers to explore some different ways to improve their market penetration. Though Edible and biodegradable films are not meant for total replacement of synthetic packaging films (Krochta 1997), but the application of these films permit very diverse objectives such as the control of moisture loss, microbial growth, preservation of the structural integrity of the product or gradual release of antioxidant and antimicrobials into the food and food products (Arvanitoyannis *et al* 1996).

The suitable use of edible packaging strongly depends on their mechanical and barrier properties and are very promising systems for the future improvement of food quality and preservation during processes and storage.

Edible films are fit for human feeding but may or may not be biodegradable in nature. However, biodegradable films may or may not be fit for human consumption and is degradable by micro flora. Edible packaging is not other than traditional packaging; however it provides new approach against traditional stress factor to be applied for food preservation. It also helps to reduce the cost and the amount of traditional packaging used. These are natural polymers obtained from agricultural productions such as animal and vegetable proteins, gums, and lipids and are perfectly biodegradable, and therefore perfectly safe for the environment. These can control moisture, gases and lipid migration and can be a supporter of additives and nutrients. Water, ethanol and combination of both are used as solvent for the production of edible films. Researchers tried to develop and apply edible films made from variety of agriculture commodities and waste of food product industrialization. Such material includes polysaccharides, proteins and their blends. Like synthetic packaging, these materials can also be the carriers of different additives such as antioxidants, antimicrobials, neutraceutical and flavouring agents. Starch, cellulose derivatives, chitosan/chitin, gums, lipid, and proteins offer the possibility of obtaining edible films in fresh or processed food packaging to extend the food's shelf life. Amongst these, chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible in addition to having antimicrobial characteristics (Darmadji and Izumimoto 1994, Jayakumar *et al* 2007).

Edible packaging is a kind of intelligent packaging because they are both active and selective and have infinite potential use. Their cost is 10-to50-fold higher than those of polyethylene or polypropylene films, but is complex, multilayered, or active plastic films. However, their cost is not a handicap to their development because quantities used are very low, and they are especially applied for very specific goals in value-added food products. Thus, the knowledge of edible polymers and that of plastic materials should be used synergistically for the development of new applications, new biodegradable materials, and new environmental approaches. Consequently, edible packaging has potentiality appear to be a successful key for tomorrow's food packaging.

Edible films made with several substances have been perfected in order to account for the complementary functional properties of each component and to

minimize their disadvantages. The film-forming substances are able to form a continuous structure by settling the interactions between molecules under the action of a chemical or a physical treatment. Edible films and coatings have been applied on meat, poultry, seafood, fruits, vegetables, grains, candies, heterogeneous and complex foods, or fresh, cured, freezed, and processed foods.

Starch has been evaluated in its film forming ability for applications in the food packaging area; characteristics of the starch film matrices, film formation methods, and physico-chemical properties of the starch films (Jimenez *et al* 2012). Edible or biodegradable starch films can be obtained from the native starch or its components, amylose and amylopectin, by two main techniques: solution casting and subsequent drying (wet method) and thermoplastic processing (dry method) (Paes *et al* 2008). Modified (Lopez *et al* 2008) and soluble or pre-gelatinized starch have also been used (Pagella *et al* 2002) to obtain starch films in many studies. Many researchers have obtained films from different starch sources in combination with plasticizers. Films based on starch are transparent (Mali *et al* 2004), odorless, tasteless, and colorless. Edible films appear to be a complementary parameter, interesting, and sometimes essential for the quality and stability of some fresh, treated, or frozen food products. Because they are both a packaging and a food component, edible films and coatings have to fulfill some requirements:

- Good sensory qualities
- High barrier and mechanical efficiencies
- Enough biochemical, physico-chemical and microbial stability
- Free of toxics and safe for health
- Simple technology
- Non-polluting
- Low cost of raw materials and process.

Till date, lot many studies have been undertaken on edible packaging of food and dairy products, but studies pertaining to *khoa* based sweets is scare. Also, considering the huge demand of *khoa* based sweets i.e. 6.5 % of total milk products, widely popular milk product milk cake has been taken up for further investigation. The idea of taking up milk cake as product under investigation includes the use of butter paper in milk cake wrapping as primary package, which results in loss of some

flavour and stickiness of contents with the paper. This would also make the general consumer aware regarding the positive aspects of this newer packaging film and introduction at local market for its wider acceptability. Thus, keeping the gaps in mind, study was planned with following objectives as follows:

1. Selection and optimization of ingredients and their level for development of edible film.
2. Physico-chemical, sensory and microbiological characterization of the developed film and evaluating its various characteristic properties.
3. Comparative storage study of product quality in developed film with conventional butter paper

CHAPTER – II

REVIEW OF LITERATURE

The demand for natural foods has been increasing day by day and this has forced the industries and researchers to find some different ways to improve the market penetration by producing more products with improvement in quality, food safety and freshness (Peelman *et al* 2013). Nowadays the new trend consisting of the development of innovative biopolymers obtained from agricultural commodities and/ or food-waste products (Valdes *et al* 2014). Plastics were increased throughout the second half of the 20^{th} century. There use in market is more due to their relatively low price, mechanical resistance, heat seal ability, shape versatility, and degrees of rigidity, but these are non-renewable and non-degradable. Actually recycling of the plastics is very limited due to some technical and economic issues.

Incineration of the plastics produces toxic compounds, such as furans and dioxins produced by burning polyvinylchloride (PVC) (Jayasekara 2005). The accumulated waste generated by the disposal of petroleum-derived polymers has become the headache nowadays as they have dangerous effects on the environment. This has become the worldwide demand for replacing conventional plastics by renewable and biodegradable polymers in the last decades (Babu 2013).

The use of biopolymers in multiple food-packaging applications has emerged as an alternative with regard to their film-forming properties to produce edible films as an environmentally friendly technology (Espitia 2014). Starches, cellulose derivatives, chitosan/chitin, gums, animal or plant-based proteins, and lipids are some of the sources to obtain edible films in fresh or processed food packaging to extend the food's shelf life. These polymers have some additional benefits in their commercial use, such as biocompatibility, barrier properties to moisture and/or gases, non-toxicity, non-polluting characteristics, mechanical integrity, and low cost (Sadaka 2013, Silva-Weiss 2013). Edible films can be used as carriers for antioxidant/ antimicrobial additives to extend food's shelf life by maintaining their mechanical integrity and handling characteristics (Fani 2015). It should be necessary that the edible films should have only food-grade components in their compositions not only the film-forming matrix, but also the solvent, plasticizers and any other additives. Many of functions of edible films are somewhat similar to those of synthetic

packaging materials and should replace the synthetic packaging which would help to reduce the environmental impact of the massive use of synthetic plastics.

2.1 Film Formation

The native starch or its components, amylose and amylopectin are the main sources from which the edible or biodegradable starch films are obtained. There are two main techniques as stated by Paes *et al* (2008) through which the films were obtained are solution casting and subsequent drying (wet method) and thermoplastic processing (dry method). Lopez *et al* (2008) revealed that modified and soluble or pre-gelatinized starch has also been used to develop the starch films. Another method from which the starch films may be obtained is a dry process (thermoplastic or thermal processing) in which the water content is lower compared to the wet process. A dry process can be used for the materials which have thermoplastic properties such that these become soft (melted or rubbery) at a temperature lower than decomposition temperature and they can change their shape according to the mold under a thermal/mechanical process.

According to a research by Carvalho (2008), to use native starch to obtain film, the granules should be distrupted by the gelatinization process in an excess of water media (>90 % w/w). In starch gelatinization process, there is a granules swelling, depending on the available water, provoking the breakage of the amylopectin matrix and releasing the amylose.

Actually, the gelatinization process initiates at low temperatures and continues until the granules are completely disrupted (Ratnayake and Jackson 2007). Researchers studied seven types of starch by scanning electron microscopy (SEM) and observed effect of treatment at different temperatures and reported the granule structure and further described the gelatinization as a three-stage process during which different structural events take place:

1. The absorption of water by starch granules led to increase in starch polymer mobility in the amorphous regions.
2. Starch polymers in the amorphous regions rearrange often forming new intermolecular interactions.
3. With increasing hydro-thermal effects, the polymers become more mobile and lose their intermolecular interactions and overall granular structure.

2.1.1 Wet Process

The wet process consists of forming a film by means of dispersion or as an emulsion. The film forming dispersion is generally poured on a leveled surface like a petri dish (Bertuzzi *et al* 2007[b]) or a Teflon® plate (Jiménez *et al* 2012) to obtain films by casting. The complete process (from native, modified, or pre-gelatinized starch to final film) could be divided into several steps: gelatinization and dispersion, homogenization of the mixture (in the case of emulsions or mixes), casting, and drying.

Srichuwong *et al* (2005) observed that the first stage is always the gelatinization of the starch, or dispersion if the pre-gelatinized kind is used. The process is optimized according to the origin of the starch; therefore the granule structure depends on the starch source. In most cases, heating is used to mix the starch with other materials prior to gelatinization. These materials may affect the gelatinization process, which has to be kept in mind, when the dispersions are prepared.

Tan *et al* (2004) in a study revealed that the gelatinization temperature varies, as the glycerol content increases. However, there was no effect on gelatinization temperature, when triglycerides or fatty acids were added to various types of starch in different conditions. Usually, the starch granules are disrupted by means of a heating step and it is also possible to achieve a similar result using an alkaline medium. This method of granule disruption is called cold gelatinization.

The films from quinoa (Chenopodium quinoa) starch were obtained in which the starch was processed from a quinoa starch powder and treated with NaOH to initiate the disruption of starch granules during the gelatinization process, which was carried out at 97 °C for 30 min (Araujo Farro *et al* 2010). Bertuzzi *et al* (2007[a]) investigated a gelatinization step preceded by different periods of alkaline treatment and observed its effect on the final properties of high-amylose corn starch films. They observed that the use of combined starch disruption uses low gelatinization temperature instead of using traditional high temperature process.

Romero-Bastida *et al* (2005) compared different properties of the films obtained by both cold and thermal gelatinization. Authors used non-conventional starch sources, such as banana, mango, and okenia, to compare the structural, mechanical, and water vapor barrier properties of films. According to SEM

observations, there was a homogeneous matrix when hot gelatinization taken place. On the other hand, cold gelatinization led to a cracked structure. This observation resulted the poorer water barrier and mechanical resistance shown by cold gelatinization obtained films.

After the gelatinization process and there are other components such as glycerol and other compounds have been added to the mixture, the following step is homogenization. This step can be ignored depending on the properties of components in the film-forming dispersion. There are many cases in which films were obtained from different starch sources in combination with plasticizers in which, a homogenization step was not necessary.

Paes *et al* (2008) concluded that there is no agreement on a standard method for the preparation of starch films to obtain the required functional and physico-chemical characteristics. Therefore, the preparation of starch films is a process which depends on several factors, such as the type of starch and plasticizer, and must be optimized in order to obtain films with adequate properties.

2.1.2 Dry Process

Carvalho (2008) described Thermoplastic Starch (TPS) as an amorphous or semi-crystalline material composed of gelatinized or destructurized starch containing one or a mixture of plasticizers. TPS can be softened and hardened so that it can be molded shaped by the heat and shear forces. Liu *et al* (2009) analyzed that, starch can be processed thermally by sheet/film extrusion, foaming extrusion, injection molding, compression molding, and reactive extrusion. However, without physical forces, the gelatinization process depends on water content and temperature conditions. Whereas in extrusion or thermal processes, gelatinization is typically achieved at low moisture content due to the high-shear and high-pressure conditions, which distrupt the starch granules, allowing faster water transfer into the starch molecules (Burros *et al* 1987).

Starch based films can obtained by means of thermal processes includes two main steps: Firstly, the starch is mixed with plasticizers and extruded in order to disrupt the starch granules. Another step is thermo mold the obtained paste to form film. Averous and Boquillon 2004 explored that as the starch reaches to an amorphous state, it can be injection-molded, extruded with a film-blowing die (Thunwall *et al* 2008), or thermo pressed (Chung *et al* 2010; Muller *et al* 2011).

Pushpadass *et al* (2008) analyzed the effect of the temperature (110 or 120 °C) in the sheeting die on the different properties of the final product and found significant, temperature-dependent differences. This study mainly focused on the structural changes which occur in the polymer when subjected to temperature or mechanical actions to better understand the dry process.

2.2 Classification according to the components

To simplify, the review has been divided into sub heads to acknowledge different areas of packaging studies undertaken by different authors.

2.2.1 Starch based edible films

Parra *et al* (2004) prepared the edible film using cassava starch. The crosslinking agents were the blends of glycerol (GLY) and polyethylene glycol (PEG) as plasticizers, and glutaraldehyde (GLU) and which were used determine the mechanical properties and water vapor transmission of those films. A response surface methodology was applied on the results to identify the blend with the best mechanical properties and lowest water vapor transmission. Authors observed the crosslinking effect of glutaraldehyde in the films whereas plasticizing action of polyethylene glycol was restrained by more than 0.5 g of glutataraldehyde. The use of glycerol was less evident for this property even after 284 h of contact time with water vapor.

An endeavor to prepare edible films made of agar (AG), cassava starch (CAS), normal rice starch (NRS), and waxy (glutinous) rice starch (WRS) was done by Phan *et al* (2005). They analyzed the functional properties of edible agar-based and starch-based films for food quality preservation. The water vapor permeabilities (WVP) were comparable with most of the polysaccharide based films and protein-based films. They found when the relative humidity (RH) is greater than 84%, the WVP of the films varies and remains constant depending upon the environmental moisture pressure. Among these polysaccharide-based films, AG-based film and CAS-based film had good mechanical properties: being transparent, clear, homogeneous, flexible, and easily handled. NRS- and WRS-based films were relatively brittle and have a low tension resistance. Microstructure of film cross section was observed by environmental scanning electron microscopy to better understand the effect of the structure on the functional properties. The results suggested that AG-based film and

CAS-based films, showed better functional properties, are promising systems to be used as food packaging or coating instead of NRS- and WRS-based films.

A study conducted by Mali *et al* (2006) explored the possibility of using corn, cassava and yam starch films for the preparation of edible film. They analyzed the thermal, mechanical and barrier properties of corn, cassava and yam starch films and the behavior of these three starches films under a controlled storage (64% RH and 20 °C). During storage, the increase in crystallinity was higher in unplasticized than in plasticized films. Thus, unplasticized stored samples become more brittle and less permeable during storage. The values decreased when glycerol content reached at 20 g/100 g starch because a more compact structure was formed and at 40 g glycerol/100 g starch, WVP increased owing to dense film matrixes.

Rodriguez *et al* (2006) studied the combined effect of plasticizers and surfactants on the physical properties of starch based edible films. Films were made up of potato starch, glycerol and Tween 20, Span 80, and soy lecithin (as surfactants). Films were characterized according to water vapor permeability (WVP) and mechanical properties. The addition of plasticizers resulted in more flexible and manageable films and higher WVP. A synergistic behavior between the plasticizer and the surfactants was observed. Films with glycerol and high level of any of the surfactants behaved as films with lower tensile strength, higher elongation, and higher WVP. The most intense synergistic effect with glycerol was showed by the Tween 20, as well.

The objective of the study set by Bertuzzi *et al* (2007[b]) was to prepare and characterize the starch based film. At low temperature, with the reduction in gelatinization temperature by an alkaline pretreatment of starch, high amylose corn starch (HACS) based films were obtained. Films were physically and chemically characterized through film solubility in water, water sorption isotherms, opacity and crystallinity of films. The film properties were affected by alkaline treatment time of HACS to gelatinization. It was found that when it reached to asymptotic value after treatment time of 60 min, film solubility and opacity decreased while crystallinity increased. Above 30% of glycerol, an increase in plasticizer content led to a loose network, as a result film opacity and water sorption increased.

Another study was carried out by Talja *et al* (2007) on evaluation of developed Potato-Starch-Based edible films. The authors analyzed the effect of

amylose content on physical and mechanical properties of potato-starch-based edible films. X-ray diffraction determined the crystallinity of selected native starches and edible films made of the same starches. The amylose content of potato starches varied between 11.9 and 20.1%. Gelatinization of potato starches in excess water occurred at temperature ranging from 58 to 69 °C independently of the amylose content. The native potato starches with low, medium, and high amylose content contained 10-13% relative crystallinity. Instead, films prepared from the same potato starches were found to be amorphous having the relative crystallinity of 0–4%. The mechanical properties and the water vapor permeability of the films were found to be independent of the amylose content.

Talja *et al* (2008) assessed the different aspects of starch-based edible films and analyzed the effect of type and content of binary polyol mixtures on physical and mechanical properties of starch-based edible films. Researchers investigated the effects of binary mixtures (1:1) of glycerol, xylitol and sorbitol at various concentrations on physical and mechanical properties of potato starch-based edible films stored at various relative vapor pressures (RVP). Results indicated that, as the content and plasticization effect of binary polyol mixture as well as RVP gradient increased, Water vapor permeability (WVP) of films also increased. However, when films were plasticized at the high content of binary polyol mixtures and stored at the RVP of 54% and 76%, tensile strength and elongation at break were decreased. Crystallization of polyols was not observed when binary polyol mixtures were used as plasticizers.

The oxidized potato starch (OPS) films were prepared by Hu *et al* (2009) and analyzed the characteristics of the films. The mechanical properties of these films were measured, and it was observed that the film with 19.4% glycerol exhibited the desirable mechanical properties. X-ray diffraction study showed an increase of glycerol content led to a decrease in the crystallinity for OPS films, and storage conditions such as storage time, storage temperature and relative humidity also had certain effects on the retro gradation of starch owing to re-crystallization. OPS films had excellent anti-leakage ability for vegetable oil, good anti-crosslinking ability in saturated formaldehyde vapor, and good stability in acid aqueous medium, but poor stability in alkali aqueous medium.

Edible films developed from potato peel were investigated for different film properties, including moisture barrier and tensile properties, color, and microstructures, from the films formed with different concentrations of plasticizer (glycerol) and emulsifier (soy lecithin). With decrease in the concentrations of glycerol and soy lecithin by 40 and 75%, respectively, water vapor permeability (WVP) of films decreased by 32%. The tensile strength of the films decreased up to 71% and the elongation at break increased up to 161%, when the concentration of glycerol or soy lecithin increased, demonstrating lubricant effects of glycerol and soy lecithin. Also, Kang *et al* (2010) from an investigation presumed that the concentrations of glycerol and soy lecithin were found to be the important variables in producing biopolymer films from potato peel.

Ghanbarjadeh *et al* (2011) prepared corn starch based film for improving the barrier and mechanical properties of film. Researchers also studied the effect of citric acid and carboxy methyl cellulose (CMC) and investigated that the films produced from pure starch are brittle and difficult to handle. With the chemical modifications (e.g. cross-linking) and using a second biopolymer in the starch-based composite had been studied as strategies to produce low water sensitive and relatively high strength starch based materials. Corn starch films with varying concentrations of citric acid (CA) and carboxy methyl cellulose (CMC) were produced by casting method. The effects of CA and CMC on the water vapor permeability (WVP), moisture absorption, solubility and tensile properties were investigated and revealed that increase in CA percentage from 0 to 10% (W/W), the water vapor barrier property and the ultimate tensile strength (UTS) improves significantly ($p < 0.05$).

Bonilla *et al* (2013) uncovered a research, which states that the development of wheat starch glycerol films and studied the properties of film on chitosan addition. To evaluate of the impact of chitosan on the physical properties of wheat starch–glycerol films, part of the wheat starch was replaced with chitosan, and the effect of composition on the properties of both the films and the film-forming dispersions was studied. The mechanical and barrier properties of the films were affected by the combined effect of the glycerol and chitosan. The antimicrobial properties of the films were directly affected by chitosan ratio, which showed a significant bactericide activity when the chitosan–starch ratio in the film was 50%.

A research was carried out on the development of edible films using native wheat starch using different concentrations of glycerol (0, 20, 30, 40 and 50% of starch dry weight basis), (Farahnaky *et al* 2013). The effects of glycerol on the microstructure, crystallinity, solubility in water, moisture absorption, water vapor permeability, optical and mechanical properties of the films at 25ºC and relative humidity range of 11–84% was investigated. The lowest water vapor permeabilities were found for the films with 20 and 30% glycerol. However, it did not changed X-ray patterns of starch films; whereas, the degree of crystallinity was reduced. All starch films stress at break and Young's modulus decreased and elongation increased when glycerol concentration and/or RH increased.

Gutierrez *et al* (2015[a]) assessed the different aspects related to edible films from corn starch. The goals of this study were to elaborate and characterize starch corn films 80:20 "waxy", regular, from native and modified (cross-linked), to define their potential application. It was resulted that, films with modified starch had highest hydrophilic properties which increased its thickness, permeability and solubility, and with major stability in acidic and alkaline medium. Finally, physico-chemical properties and water vapor barrier properties of the films resulted the strong interaction phosphated starch-plasticizer.

As reported by Gutierrez *et al* (2015[b]) films made from phosphated starches were more hydrophilic, producing an increase in solubility and crystallinity. Edible, bio-degradable films based on native and phosphate cush-cush yam and cassava starches plasticized with glycerol were developed using casting method. The physico-chemical properties of each of the different starch films were then evaluated and compared in order to determine their potential applications in the food industry. Due to the amylose molecules in the cassava starch strongly interacted with glycerol which resulted in an increase in the number of hydrogen bonds, and led to increase the temperature required for the degradation of the cassava starch-based films, and even higher temperatures for degrading the films based on the modified starches. It was observed that the cassava films developed was suggested for making good packaging materials, while films derived from cush-cush yam are more suitable as food coatings.

Lagos *et al* (2015) conducted some experiments to assess the degree for usage of prepared cassava starch films. They analyzed the effect of different plasticizers (glycerol and sorbitol), and different relative humidity (43, 58, 75 and 85%)

conditions on the mechanical properties of the film. Results indicated that, plasticizer ratio directly influenced the force values of the films, when its content in formulation was increased. Sorbitol produced films which were more resistant to puncture than glycerol under low relative humidity conditions.

In this study, Fan *et al* (2016) reviewed the effects of adding different contents of starch nanoparticles (0%, 0.5%, 1%, 2%, 5%) weight% prepared by the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation (TEMPO-SNPs) on the properties of maize starch films. Differential scanning calorimetry (DSC), X-ray diffraction analysis, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and texture profile analysis were used to characterize the thermal properties, morphology and structure of the prepared films. Results revealed that, water vapor permeability (WVP) of films reduced significantly from 4.21 to 3.04×10^{-8} g m^{-1} s^{-1} Pa^{-1}, when the content of TEMPO-SNPs was increased. As the TEMPO-SNPs content increased, elongation at break, tensile strength and Young's modulus of the films also increased. At the TEMPO-SNPs content of 1%, the elongation at break, the tensile strength and Young's modulus of the films peaked. Also, SEM showed that the nano-composite films had smoother surfaces and cross sections with no cracks or visible air pockets.

Seligra *et al* (2016) standardized and developed the method for the production of biodegradable and non-retrogradable starch–glycerol based films using citric acid (CA) as crosslinking agent at 75 °C. The temperature at which the gelatinization process was carried out was critical to obtain the best results. An increase of gelatinization process temperature at 85 °C in system with CA, led to a worsening on WVP and its integrity after a swelling process with dimethylsulphoxide (DMSO), compared to the films processed at 75 °C.

2.2.2 Protein Films

Kim *et al* (2001) developed plasticized whey-protein and whey-protein emulsion films using sorbitol and glycerol as plasticizers and butterfat and candelilla wax as lipids. Authors evaluated that the Plasticizer and lipid incorporation as influenced by the solubility and equilibrium moisture contents (EMC) of the films. Results revealed that EMCs of all films increased rapidly at $a_w \geq 0.65$. Incorporation of lipids reduced solubilities and EMCs of sorbitol and glycerol plasticized films.

In a study, Perez-gago *et al* (2001) prepared the whey protein film and observed that the solubility of whey protein film was decreased as film forming solution heating time and temperature increased. When time and temperature of film forming solution was increased, films became stiffer, stronger and more stretchable. Oxygen permeability (OP) was lower for films made from heat denatured whey protein than for films made from native whey protein. Results suggested that there was increase in covalent cross linking, as heat denaturation of the whey protein increased; this was accountable for film water insolubility, higher tensile properties and lower OP.

Gounga *et al* (2007) proposed a study to evaluate the edible films prepared from whey protein isolate (WPI), and characterized to optimize the best combination of protein concentration and glycerol. It was resulted that, WPI:Gly ratio showed the best combination with factors considered being thickness and water vapor permeability (WVP), while the 9% WPI with 3.6:1 WPI:Gly showed the best result as seen from the oxygen permeability (OP). They also conducted the further study by adding pullulan (PUL) at different to a selected film in order to investigate the effect of pullulan on thickness, OP, WVP, moisture content (MC), film solubility (FS) and morphology using scanning electron microscopy (SEM). WPI–PUL film had a good appearance and 1:1 WPI:PUL resulted in films with greatest values of OP, WVP, MC, FS, and transmittance, whereas, the SEM micrographs showed the low barrier ability. However, the properties were modified with addition of PUL at low concentration, hence improving the potential characteristics of WPI-based films for food applications.

The individual and interactive effects of glycerol and chitosan on tapioca starch-based edible film properties were investigated by Chillo *et al* (2008) using response surface methodology. Tests were run on the polymeric matrices to determine film forming solution apparent viscosity, mechanical and dynamic-mechanical properties, water vapour permeability (WVP) and color. All film forming solutions exhibited pseudo-plastic behaviour. It was observed from the mechanical characteristics point of view, that the chitosan had a positive effect while the glycerol had a negative effect. The $\tan \delta$ values were affected more by glycerol than the chitosan. With regards to WVP data, the chitosan addition had a negative effect, whereas the glycerol one had a positive influence. Moreover, both the chitosan and

glycerol influenced the color indices. It can be concluded that the concentrations of chitosan and glycerol led to changes in tapioca starch edible film properties, potentially affecting film performances.

Javanmard *et al* (2008) developed the edible films and coating from whey protein, a by-product of cheese manufacture, and which acted as oxygen, aroma, oil and/or moisture barriers. For this, they packed the dried peanut kernels into whey protein concentrate (WPC)–olive oil composite films and stored at 25°C for 4 weeks. Peroxide value (PV), moisture uptake and sensory attributes of control (unpacked) and packed samples were evaluated for 28 days. It was observed that maximum moisture uptake was found (5.40±0.14%) in unpacked (control) peanut kernels, but packaging peanuts in WPC–olive oil films led to minimum moisture uptake. WPC–lipid composite films can delay the development of rancidity of dried peanuts at intermediate (50%) relative humidity. Incorporation of oil in WPC films had no significant effect ($p>0.05$) on the PV of packed peanuts. Weight loss of unpackaged peanuts at a higher level (10.15% w/w) were significantly bigger than peanuts packaged at a lower level (1.05% w/w). These results suggested that a whey protein based film is a viable alternative packaging process for peanuts and could improve the shelf life and some sensory properties of nuts.

Ozdemir *et al* (2008) studied the impact of protein, sorbitol, beeswax and potassium sorbate focuses in whey protein films on their water vapor penetrability, water dissolvability and organoleptic properties using mixture response surface methods. All components including protein, sorbitol, beeswax and potassium sorbate affected water vapor permeability and water solubility of the film. Beeswax was the most imperative factor affecting the stickiness and appearance of the film. Measure of protein (50– 65%, w/w) had no impact on stickiness and appearance, while the measure of sorbitol (35– half, w/w) in the films had no effect on appearance. Blend extents of protein = 0.53, sorbitol = 0.38, beeswax = 0.08 and potassium sorbate = 0.01 would yield a consumable film with least stickiness, water vapor penetrability $\leqslant$ 9 g mm m^{-2} h^{-1} kPa^{-1}, water solubility $\geqslant$ 39% and appearance score $\geqslant$ 80.

Pierro *et al* (2011) evaluated the effect on shelf life extension of Ricotta cheese covered with a chitosan/whey protein edible film and stored under modified atmosphere at 4 °C. Author observed that, the chitosan/whey protein film had 35% and 21% lower oxygen and carbon dioxide permeability and around three times

higher water vapor permeability than film arranged with chitosan alone. Over a 30-day storage period, no difference in the pH of control and covered Ricotta cheeses was observed. While the titratable acidity of the control expanded linearly for the initial two weeks and stayed consistent for rest of the storage period, comparing Ricotta cheese did not change for the initial 21 days and achieved the acidity level (0.34 ± 0.02 meq/100 g of analysed sample) of the control on 30^{th} day. They suggested that a potential utility of chitosan/whey protein coatings to extend fresh dairy product shelf-life

An active protein-based film was developed by Bahram *et al* (2014) through incorporating cinnamon Essential oil (CEO) into whey protein concentrate (WPC) at a level of 0.8 and 1.5% v/v. The impacts of CEO on microstructure, physical, mechanical and antimicrobial properties of the films were examined. The water vapor permeability of the films and water solubility of WPC matrix was diminished by 38.03 and 29.4%, respectively with addition of CEO. The films containing CEO additionally showed lower affinity to water; the contact angle was extended up to 89.61% in 1.5% CEO concentration. Films containing CEO demonstrated antibacterial activity against both gram-positive and gram-negative strains, and displayed great inhibitory effect on the fungi. A heterogeneous crack structure appeared in CEO containing films which diminished their tensile strength. It was resulted that the film could be applicable on food packaging.

The study was conducted by Galus *et al* (2016) to develop whey protein isolate films modified with almond or walnut oils at low concentrations (0.5 and 1.0%) through emulsification keeping in mind the end goal to modify properties of the films. The swelling, water vapour permeability, and surface hydrophilicity of the whey protein films were reduced by the addition of the oils. Oxygen and carbon dioxide permeability were increased with an increase in the oil content. Contact angle measurements through time showed swelling effect by the control film, whereas the absorption of water droplets was showed by the emulsified films. Almond oil showed the stronger plasticizing effect and was more effective in modifying the properties of whey protein films. The results described that almond and walnut oils used at low concentrations improved the hydrophobic character of the whey protein films and have a good potential for incorporation into whey protein isolate to make edible films or coatings for some food applications.

2.2.3 Composite films

The edible starch protein-based films were assessed for the mechanical and barrier properties of film by Jagannath *et al* (2003). Authors investigated the mechanical properties and water vapor transmission behavior at different relative humidity conditions. DSC thermograms of edible films with thermal or non-thermal process were also analyzed. Results indicated that casein-based film demonstrated a lower water vapor transmission rate, water gain at different relative humidity conditions, and higher tensile strength compared to the films containing gelatin and albumin. Since the casein–starch blend gave better film properties, a blend of hydrophobic carnauba wax and casein was prepared to compare the properties of hydrophilic–hydrophilic and hydrophobic–hydrophilic blends. Whenever wax based film showed multi phased behavior in the DSC thermograms and the percent elongation was lower compared to the casein–starch blend.

Peressini *et al* (2003) analyzed the rheological properties of starch–methylcellulose based edible film-forming dispersions. These properties of edible film-forming dispersions containing corn starch, methylcellulose (MC) and glycerol were studied using oscillatory and steady shear flow tests. The combined effects of glycerol content and blending levels of MC with starch on the rheological properties of dispersion were evaluated. The flow curves showed shear-thinning behavior. Dispersion stability results revealed total recovery of the viscoelastic properties of dispersions subject to high strains, as expected for entangled polymers. MC was the main factor influencing apparent viscosity and viscoelastic properties.

Another study by Bravin *et al* (2006) investigated the effect of deposition process used for film-forming dispersion (spreading and spraying), relative humidity gradient across the film (from 22–65% to 22–85%) and film thickness (15–90 μm) on mechanical properties of edible film composed of corn starch, methylcellulose (MC) and soybean oil. The effectiveness of edible coating in controlling moisture transfer in moisture-sensitive products was evaluated by a low a_w-type cereal food, coating crackers. Authors reported that spread film compared with sprayed film gave better water vapor barrier and mechanical properties. Film thickness of 30 μm was identified as optimum for the application of edible coating to bakery products. The storage of coated and uncoated (reference) crackers were done at 65%, 75% and 85% relative humidity. Moisture uptake and resistance to water vapor transmission (r) were then

calculated. Coated crackers had longer shelf-life and higher than reference at all storage conditions.

Zhong *et al* (2008) developed the edible and preservative films from chitosan/cassava starch/gelatin blend plasticized with glycerol and analyzed the physico-chemical properties of films. Edible films from chitosan, cassava starch, and gelatin plasticized with glycerol were developed by casting method. The effects of cassava starch (50, 100 and 150 g per 100 g of chitosan), gelatin (0, 25 and 50 g per 100 g of chitosan) and glycerol (21, 42 and 63 g per 100 g of chitosan) from the film solution on various properties of chitosan-based films were also studied using response surface methodology (RSM). Authors indicated that, the properties of the resulting chitosan-based blends for films were greatly influenced by the incorporation of cassava starch, gelatin and glycerol. These results indicated that there was an interaction and molecular miscibility among the major components. The growth inhibition of phytopathogen on mango fruit surface indicated the efficiency of these coatings and can be applied for the conservation of fresh or minimally processed fruits and vegetables.

Hassan *et al* (2012) visualize the effect of plasticizers on the mechanical properties and water vapor permeability of the film. Physical and mechanical properties of edible films based on blends of sago starch and fish gelatin plasticized with glycerol or sorbitol (25%, w/w) was assessed. Film forming solutions of different ratios of sago starch to fish gelatin (1:0, 2:1, 3:1, 4:1, and 5:1) were prepared and cast at room temperature. Wherein, amylose content of sago starch was between 32 and 34 per cent and the protein content of the fish gelatin was found as 81.3 per cent. The findings of this study showed that the addition of fish gelatin in starch solutions has a significant effect ($p < 0.05$), resulting in films with lower tensile strength (TS) and higher water vapor permeability (WVP). The morphology study of the sago starch/fish gelatin films showed smoother surfaces with decreasing protein in the samples with either plasticizer. DSC scans showed that plasticizers and protein content incorporated using sago starch films reduced the glass transition temperature (T_g) and melting temperature (T_m) and the melting enthalpy (ΔH_m). In this study, observation of a single T_g is an indication of the compatibility of the sago starch and fish gelatin polymers to form films at the concentration levels used.

The aim of study conducted by Fakhauri *et al* (2015) was to develop **t**he edible films and coatings based on starch/gelatin. Researchers analyzed the film properties

and effect of coatings on quality of refrigerated red crimson grapes. They also evaluated physico-chemical properties (thickness, solubility in water and acid, water vapor permeability, opacity, tensile strength and elongation at break) of composite films based on corn starch (native, modified waxy or waxy) and gelatin, plasticized with glycerol or sorbitol. After this, the formulation presenting the physico-chemical properties more appropriate was applied as an edible composite coating onto Red Crimson grapes to extend the shelf-life. The addition of gelatin significantly increased mechanical strength, solubility in water, permeability to water vapor, and thickness of the biofilms, while also decreasing the opacity. Composite films prepared with sorbitol had significantly lower permeability to water vapor and higher tensile strength than the films plasticized with glycerol. Improved appearance was observed in coated grapes after 21 days storage under refrigerated conditions, which had lower weight loss than the control group. Sensory evaluation showed that all the coatings did not affect acceptability scores.

The objective laid by Rubilar *et al* (2015) for this research was to study the effect of the film microstructure of oil-in-water emulsions stabilized by hydroxypropyl methyl cellulose/whey protein isolate (HPMC/WPI) with or without sodium dodecyl sulfate (SDS) over physical properties of HPMC/WPI emulsion-based films. The films were prepared with different HPMC/WPI-oil-SDS combinations and its physical properties were evaluated. The results showed no statistical differences ($p > 0.05$) between the thickness of edible films (0.156 ± 0.004 mm). The effect of oil content and incorporation of SDS showed the inverse trend for WI and ΔE, the increasing order of change, for WI and ΔE, among the formulation evaluated was: HPMC/1WPI-1 > HPMC/2WPI-0.5 > HPMC/2WPI-1.0-SDS ≈ HPMC/1WPI-0.5-SDS ≈ WPI > HPMC for WI and HPMC/1WPI-0.5-SDS > HPMC/2WPI-1.0-SDS > HPMC/2WPI-0.5 > HPMC/1WPI-1 for $\Delta \boldsymbol{E}$, respectively.

2.2.4 Antimicrobial films

Eswaranandam *et al* (2004) analyzed the partial replacement of glycerol with citric, lactic, malic, and tartaric acids on the antimicrobial activities of nisin (205 IU/g protein) incorporated in soy protein film against *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella gaminara* inoculated into 2.6% malic acid incorporated films and lactic acid incorporated films with nisin (5.7 and 3.4 log number colony

forming units (CFU)/mL, respectively) and without nisin (3.2 and 3.0 log number CFU/mL, respectively) had fewer survivors than HCl incorporated film with and without nisin (8.6 and 7.9 log number CFU/mL, respectively). Malic acid (2.6%) incorporated soy protein film had the fewest survivors of *L. monocytogenes*, *S. gaminara*, and *E. coli* O157:H7 (5.5, 3.0 and 6.8 log number CFU/mL, respectively) and has the potential to inhibit a wide spectrum of microbes in product application.

Du *et al* (2008) uncovered a research in which they prepared edible film from tomatoes containing carvacrol, the main constituent of oregano oil, to extend the shelf life of the product and to reduce the risk of pathogen growth on the food surface. The main aim of this study was to evaluate the antimicrobial activities, storage stabilities, and physical, chemical and mechanical properties of novel edible films. Results in the form of HPLC analysis of the films indicated that the carvacrol concentrations and bactericidal effect of the films remained unchanged over the storage period of up to 98 days at 5 and 25 °C. Carvacrol addition to the tomato puree used to prepare the films increased water vapor permeability of tomato films. This validated the potential to prevent adverse effects of contaminated food and promote human health associated with the consumption of tomatoes.

Gemili *et al* (2009) conducted a study in which, antimicrobial packaging materials were developed by incorporation of lysozyme into cellulose acetate films. The film prepared with 5% cellulose acetate solution and 1.5% lysozyme gave the highest release rate, soluble lysozyme activity and antimicrobial activity. The porosity of the films was decreased, as cellulose acetate content was increased in casting solution and hence, reduced the release rate, maximum released lysozyme activities and the antimicrobial activities of the films. Also, lysozyme addition did not cause significant reductions in tensile strength and elongation at break values except in films prepared with 15% cellulose acetate. This study showed the good potential of asymmetric cellulose acetate films to achieve controlled release in antimicrobial packaging.

2.3 Coating

2.3.1 Polysaccharides based coating

Cerquira *et al* (2009) in their investigation applied the polysaccharides from different non-traditional sources i.e. Chitosan, galactomannan from *Gleditsiatria canthos*, and agar from *Glacilaria birdiae* with different formulations along with the

addition of plasticizer and corn oil for cheese coatings. They analyzed the surface properties of the cheese and the wetting capacity of the coatings on the cheese. Three best solutions for each polysaccharide were optimized and the films were cast and water vapor, oxygen, and carbon dioxide permeability was determined, along with opacity. The O_2 consumption and CO_2 production rates of the cheese with and without coating were evaluated, which showed decrease in the respiration rates when the coating was applied. However, the uncoated cheese had an extensive mold growth at the surface compared to coated cheese. Through this, one could conclude that these coatings can be applied as an alternative to synthetic coatings.

Cerquira *et al* (2010) carried out a study on evaluation of the effect of two different coatings (galactomannan and chitosan) and storage temperature on the gas permeability "Regional" cheese. They analyzed that the coating in cheese samples was used to decrease the water loss and the colour changes during the storage time. The coating decreased the loss of moisture of the cheese in 2.5% and 1.9%, and the weight loss in 3.8% and 3.1% at 4 °C and 20 °C, respectively. Also, the hardness of the cheese decreased with coating as the storage temperature got decreased. It was observed that temperature range (4–20 °C) had a statistically significant effect in moisture loss, colour change, hardness and total mesophilic bacterial growth. Overall, galactomannan coating could be used to improve "Regional" cheese shelf-life as it decreases RO_2 and RCO_2, improves its weight and appearance and can be used to incorporate natural preservatives to reduce post contamination.

2.3.2 Antimicrobial coatings

The inhibitory activity of Chitosan based edible coatings was assessed by Coma *et al* (2003) against 2 food pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*) and 1 strain involved in food alteration (*Pseudomonas aeruginosa*) on model agar medium and on a real cheese food product. Numeration on model agar medium showed 100% inhibition of the development of selected Gram positive bacteria and 77% inhibition on *Pseudomonas* growth. Chitosan is thought to act through binding to the cytoplasmic membrane surface, and it is possible that the outer membrane protects the Gram negative cells. Edible chitosan coating could thus be used to increase the microbial lag phase while decreasing the maximum density of selected microorganisms and could have potential application for dairy products preservation.

Antimicrobial effects of whey protein isolate (WPI) films and coatings incorporating the lactoperoxidase system (LPOS) against *Listeria monocytogenes* was studied by Min *et al* (2005) through turbidity, plate counting, disc covering, and disc surface spreading tests using various growth media. Survival of *L. monocytogenes* applied to smoked salmon before or after the coating was monitored immediately after application and during storage at 4 °C and 10 °C for upto 35 days. The WPI coatings incorporating LPOS prevented the growth of *L. monocytogenes* in smoked salmon at 4 °C and 10 °C for 35 d and 14 d, respectively. However, the tensile properties, oxygen permeability, and color of WPI films were not significantly changed by incorporation of LPOS ($P > 0.05$).

Yildirim *et al* (2006) studied the effect of casein-coating on some properties of Kashar cheese and its effectiveness in carrying natamycin to prevent mould growth. Five cheese groups were prepared: no-coating (A), vacuum-wrapped (B), coated with casein (C), coated with casein containing natamycin (D) and dipped in natamycin solution (E). While samples A and C had significant mould growth after only one week, no visible mould growth was detected on the surface of sample D for about one month whereas Sample E showed mould growth after just three weeks of ripening probably due to the presence of non-treated areas on the cheese surface. Sample B had no visible mould growth throughout the ripening period. Sensory evaluation and electrophoretic analyses showed non-significant differences among cheese samples. These results indicated that casein-coating with natamycin can suppress mould growth for about one month without any adverse effects to cheese quality.

An investigation revealing the effects of coating with low molecular weight chitosan and high molecular weight chitosan on the decay of Murcotttangor and the maintenance of its quality was conducted by Chien *et al* (2007). 0.1 per cent LMWC coating substantially slowed the decay of Murcotttangor stored at 15 °C in comparison with a control sample and reduced decay by over 20% as compared to the fungicide TBZ. It was observed that 0.2% LMWC had effective antifungal activity and was more effective in controlling the growth of fungus on citrus fruits caused by*Penicillium digitatum* and *Penicillium italicum*. LMWC coating improved firmness, titratable acidity, ascorbic acidity and the water content for Murcotttangor stored at 15 °C for 56 days. Murcotttangor coated with LMWC exhibited greater antifungal resistance than TBZ and its quality was maintained for longer.

Companiello *et al* (2008) reported the effect of chitosan coating on fresh-cut strawberries. Manually strawberries were sliced and treated with a solution of 1% chitosan, packaged in modified atmosphere with high (80%) and low (5%) percentage of oxygen and then stored at 4, 8, 12 and 15 °C. The changes in microbiological quality and the shelf life of the samples were kinetically modeled in order to check the effects of storage temperature on the microbial indices for product quality were analyzed. The growth of microorganisms was inhibited by chitosan coating and it affected positively the stability time of the products, when the samples were packaged in modified atmosphere (with low and high percentage of oxygen). Color was affected positively in the presence of high percentage of oxygen, combined with chitosan coating. So, it was resulted that applying a chitosan coating prolonged effectively the quality and extended the shelf life of fresh-cut strawberries.

Shelf life extension of Ricotta cheese was evaluated by Martins *et al* (2010) at 4 °C with the use of edible coatings made of galactomannans from *Gleditsia triacanthos* incorporating nisin against *Listeria monocytogenes*. Three different treatments were tested in cheese: samples without coating; samples with coating without nisin; and samples with coating containing 50 $IU \cdot g^{-1}$ of nisin. To test the effectiveness of the treatments against *Listeria monocytogenes*, the surface of the cheese was inoculated with a suspension of the microorganism. Microbiological and physical-chemical analysis of the cheese samples was performed during 28 days. Results showed that the cheese coated with nisin-added galactomannan film was the treatment presenting the best results in terms of microbial growth delay ($p < 0.05$). These results demonstrated that novel galactomannan-based edible coatings, when combined with nisin, may provide consumer-friendly alternatives to reduce *Listeria monocytogenes* post contamination on cheese products during storage.

CHAPTER – III

MATERIALS AND METHODS

This chapter includes all the experimental details employed in the present study entitled, **"Development and Characterization of edible film for packaging of milk cake**", including information pertaining to the raw materials utilized along with methodologies and analytical procedures (Physico-chemical, microbiological, sensory and statistical) adopted as well as use of various equipments and instruments are mentioned herein.

The present investigation is broadly divided into Development (preparation), characterization & screening of ingredients variables & standardization of technology at laboratory scale, storage study of the optimized product. All experimental samples of investigation were prepared and analyzed in the M. Tech Dairy Technology Laboratory, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The details of the material and the methods followed are presented in this chapter.

3.1 Materials Required

3.1.1 Starch

The demand of natural or organic sources is increasing day by day for food and its packaging as well. Also such sources can replace the use of synthetic polymers. Starch has been famous for its film forming ability for applications in the food packaging industries. Starch contains two different components, amylose and amylopectin. The ratio of amylose/amylopectin depends on the source and age of the starch. Starch generally contains 20 to 25 per cent amylose and 75 to 80 percent amylopectin. Instant wheat, corn, and potato starch contain 20–30 per cent amylose, while its content in waxy starches is lower than 5 per cent and in high-amylose starches, it is as high as 50–80 per cent. There are different types of starches which can be used for making edible film such as corn, wheat, rice, cassava, potato etc. in their either normal or in modified form. Edible starches of different sources were used in the preparation of edible film in the study such as corn, wheat, rice etc. which gave different physico-chemical properties at different concentrations, with varying

mechanical properties, & further screened on the basis of their desirable characteristics.

3.1.1.1 Corn starch: Corn starch was procured from Central Drug House (P)Ltd. Product code- 030261, Batch no.- 060616.

3.1.1.2 Wheat starch: Wheat starch was procured from Central Drug House (P)Ltd. Product code- 224055, Batch no.- 010515.

3.1.1.3 Rice starch: Rice starch was procured from Central Drug House (P)Ltd. Product code- 223945, Batch no.- 010817.

3.1.2 Proteins

Protein plays an important role in the development of our body. Milk proteins such as casein, whey protein has abundant use in the metabolism of the body due its quality and high rate of absorption. Different milk proteins have been used for the preparation of edible film such as casein, whey, soy, corn zein, collagen, soy, sesame, wheat gluten, keratin and egg albumen etc. However, in the present study proteins such as edible soy protein in form of soy protein isolate and sesame protein in form of sesame protein isolate were used for the preparation of film.

3.1.2.1 Soy protein isolate: It was procured from Central Drug House (P) Ltd. Product code- 223547, Batch no.- 011534

3.1.2.1 Sesame protein isolate: Sesame protein isolate was procured from Central Drug House (P) Ltd. Product code- 225725, Batch no.- 011323.

3.1.3 Plasticizers

The use of only food grade biopolymers in the preparation of edible coatings/films gives brittle and stiff characteristics due to the excessive interactions between the polymer molecules. Plasticizers such as polyols are hydrophilic in nature, are therefore used as a supplement for the film forming materials to build the physical and mechanical properties such as to increase the flexibility of the film. The intermolecular forces and mobility of polymeric chains can be increased with interaction between plasticizers and the biopolymer, which results in improving the mechanical properties of the film. Also it has been observed that resistance of the film

to gas and vapor permeation increase with the addition of plasticizer. Following were the plasticizers tried for successful & desired characteristics.

3.1.3.1Glycerol: Glycerol used for film preparation was procured from Loba Chemie Pvt. Ltd., 107, Product code- 0015600500, Batch no.- A156101502.

3.1.3.2Sorbitol: Sorbitol was procured from Central Drug House (P) Ltd. Product code- 0243014, Batch no.- 020715.

3.1.3.3Mannitol: Mannitol was procured from Molychem, Product code- 23980, Batch no.- MCRT-12857.

3.1.4 Analytical reagents

Chemicals and reagents used in this study (ammonia, petroleum ether, diethyl ether, ethyl alcohol, potassium sulphate (K_2SO_4), copper sulphate ($CuSO_4$), Selenium dioxide (SeO_2), sulphuric acid (H_2SO_4), boric acid, Hydrochloric acid (HCl) etc) were of analytical grade and all aqueous solutions were made using glass distilled water.

3.2 Screening of sources of starch & protein

Different trials were taken with different polymers. Different carbohydrate & protein based polymers such as starches which include corn starch, wheat starch & rice starch and proteins such as sesame protein isolate & soy protein isolate along with different types of plasticizers, such as glycerol, sorbitol, mannitol were tried in this study. The level of starch and the level of plasticizer for the development of film was standardized by conducting two sub experiments. Different temperature time conditions were also tried for the drying of & finally 40°C for 16-17 hours was decided, to get desirable characteristics of film. The following table depicts various ranges of starch, proteins, plasticizer and temperature time combination for the preparation of film purpose.

Table 3.1: Development of film with different concentration of various starches and proteins with glycerol, mannitol and sorbitol as a plasticizer

Trials	Type of starch	Plasticizer (Glycerol)	Trials	Type of starch	Plasticizer (Glycerol)
1	Corn starch 4%	2%	16	Rice starch 4%	3%
2	Corn starch 5%	2%	17	Rice starch 5%	3%
3	Corn starch 6%	2%	18	Rice starch 6%	3%
4	Corn starch 4%	3%	19	Sesame protein isolate (4%)	2%
5	Corn starch 5%	3%	20	Sesame protein isolate (5%)	2%
6	Corn starch 6%	3%	21	Sesame protein isolate (6%)	2%
7	Wheat starch 4%	2%	22	Sesame protein isolate (4%)	3%
8	Wheat starch 5%	2%	23	Sesame protein isolate (5%)	3%
9	Wheat starch 6%	2%	24	Sesame protein isolate (6%)	3%
10	Wheat starch 4%	3%	25	Soy protein isolate (4%)	2%
11	Wheat starch 5%	3%	26	Soy protein isolate (5%)	2%
12	Wheat starch 6%	3%	27	Soy protein isolate (6%)	2%
13	Rice starch 4%	2%	28	Soy protein isolate (4%)	3%
14	Rice starch 5%	2%	29	Soy protein isolate (5%)	3%
15	Rice starch 6%	2%	30	Soy protein isolate (6%)	3%

Trials	Type of starch	Plasticizer (mannitol)	Trials	Type of starch	Plasticizer (mannitol)
31	Corn starch 4%	2%	46	Rice starch 4%	3%
32	Corn starch 5%	2%	47	Rice starch 5%	3%
33	Corn starch 6%	2%	48	Rice starch 6%	3%
34	Corn starch 4%	3%	49	Sesame protein isolate (4%)	2%
35	Corn starch 5%	3%	50	Sesame protein isolate (5%)	2%
36	Corn starch 6%	3%	51	Sesame protein isolate (6%)	2%
37	Wheat starch 4%	2%	52	Sesame protein isolate (4%)	3%
38	Wheat starch 5%	2%	53	Sesame protein isolate (5%)	3%
39	Wheat starch 6%	2%	54	Sesame protein isolate (6%)	3%
40	Wheat starch 4%	3%	55	Soy protein isolate (4%)	2%
41	Wheat starch 5%	3%	56	Soy protein isolate (5%)	2%
42	Wheat starch 6%	3%	57	Soy protein isolate (6%)	2%
43	Rice starch 4%	2%	58	Soy protein isolate (4%)	3%
44	Rice starch 5%	2%	59	Soy protein isolate (5%)	3%
45	Rice starch 6%	2%	60	Soy protein isolate (6%)	3%

Trials	Type of starch	Plasticizer (Sorbitol)	Trials	Type of starch	Plasticizer (Sorbitol)
61	Corn starch 4%	2%	76	Wheat starch 4%	3%
62	Corn starch 5%	2%	77	Wheat starch 5%	3%
63	Corn starch 6%	2%	78	Wheat starch 6%	3%
64	Corn starch 4%	3%	79	Rice starch 4%	2%
65	Corn starch 5%	3%	80	Rice starch 5%	2%
66	Corn starch 6%	3%	81	Rice starch 6%	2%
67	Wheat starch 4%	2%	82	Rice starch 4%	3%
68	Wheat starch 5%	2%	83	Rice starch 5%	3%
69	Wheat starch 6%	2%	84	Rice starch 6%	3%
70	Sesame protein isolate (4%)	2%	85	Sesame protein isolate (4%)	3%
71	Sesame protein isolate (5%)	2%	86	Sesame protein isolate (5%)	3%
72	Sesame protein isolate (6%)	2%	87	Sesame protein isolate (6%)	3%
73	Soy protein isolate (4%)	2%	88	Soy protein isolate (4%)	3%
74	Soy protein isolate (5%)	2%	89	Soy protein isolate (5%)	3%
75	Soy protein isolate (6%)	2%	90	Soy protein isolate (6%)	3%

3.3 Preparation of product

3.3.1 Milk cake Preparation

Milk cake is a khoa based sweet prepared from either buffalo milk or directly from *danedar* variety of Khoa. Composition of milk from which the milk cake prepared was 6.3% fat, 8.5% SNF, 0.13% acidity as lactic acid. The milk was boiled in a steam jacketed pan. When milk comes to first boil, 0.05% citric acid on milk basis was added as 1% solution. After addition of citric acid solution, small tiny granules tend to appear in milk. Heating is continued further with rigorous stirring. When the mass shows signs of leaving the surface of pan, sugar @ 50 percent of expected khoa yield or 6% on the basis of milk was added and the mix was heated with rigorous stirring. Controlled heating was required at this stage to obtain better quality product. When the product showed signs of dry appearance, the mix was poured in greased deep metal molds while hot. The milk cake was allowed to cool slowly at room temperature so that the central portion of the pat turns brown and caramelized flavor develops due to residual heat. After cooling, the product is cut into desirable sizes**.** Fig 3.1 shows the process followed in the form of flow diagram for milk cake preparation

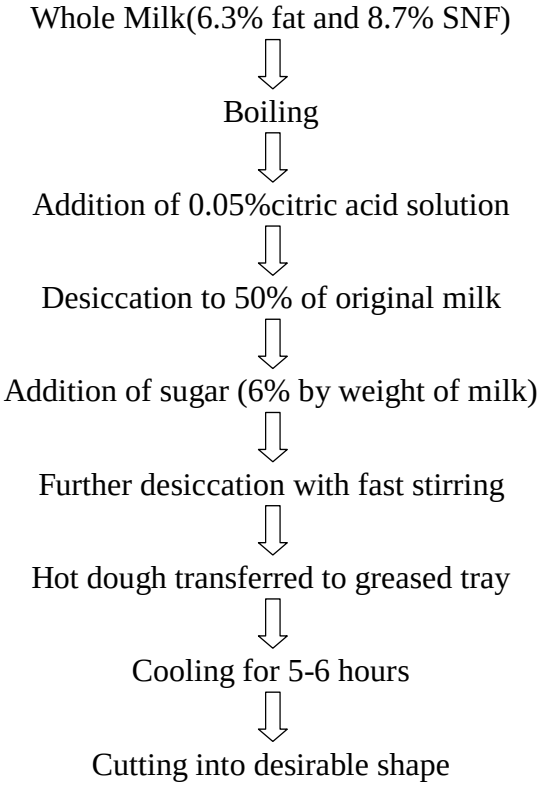


Figure 3.1: Flow sheet for Milk cake preparation

3.3.2 Preparation of edible film

Edible films were developed from a variety of sources, such as polysaccharides, proteins with the addition of other facilitating components like plasticizer to increase the flexibility of film. Edible or biodegradable starch films can be obtained from the native starch or its individual components, amylose and amylopectin, by two main methods: thermoplastic processing (dry method) and solution casting and drying (wet method). Film can be processed thermally (dry method) by sheet/film extrusion, foaming extrusion, injection molding, compression molding, and reactive extrusion method. This dry method usually includes two main steps. First, the starch is mixed with plasticizers and extruded in order to disrupt the starch granules, followed by a final step in which the obtained paste (or pellets) can be thermo-molded to form films.

On the other hand, wet method employs solvent which is required for the solution and dispersion of the polymer onto a flat surface. This follows by drying in controlled conditions for the removal of the solvent and the formation of the film. In general, wet processes could be divided into four steps: dispersion or gelatinization, homogenization, casting, and drying. The method used for the film preparation was wet or casting method. The wet method for film preparation is easy and energy efficient. Therefore this method was used, following steps for the film preparation.

3.3.2.1 Gelatinization

The first stage is gelatinization of starch or dispersion, if pre-gelatinized starch is used. This can be done by using excess water, i.e. granules got disrupted in excess water. Starch gelatinization can occur at different stages depending upon type and amount of starch and glycerol used. When starch granules are heated at 60-70°C in excess water then the crystalline structure of the granules is lost with change in temperature interval in the gelatinization temperature range. As a result, there is substantial change in the rheological behavior of starch suspension which occurs on heating. The starch granules absorb water and swelled to several times from its initial size, components of the starch granules, mainly amylose, leach out and finally a gel composed of swollen starch granules in an amylose matrix is formed.

3.3.2.2 Homogenization

Once the starch gets gelatinized, there is next step involving addition of other ingredients such as plasticizers to the mixture & is known as homogenization. Use of different types of homogenizers or instruments can be employed such as Ultra Turrax, rotar startor homogenizer, two stage high pressure homogenizer, blenders etc for the homogenization and blending of film forming solution. Ultra turrax (IKA model T 18) was used at 5000-5500 rpm for 10 minutes for the homogenization of film forming solution after mixing the ingredients and before heating. During homogenization process, air bubbles are formed in film forming suspension. These air bubbles can make micro holes in the film and these can be removed using vacuum devices. Homogenization is necessary for the mixing in order to avoid the formation of lumps and clear solution of starch so to increase the transparency of the film.

3.3.2.3 Casting

After gelatinization and homogenization process, casting is done in which the film forming solution was spread on a levelled surface. Different casting containers such as boxes, petri dishes of different material such as glass, stainless steel, polytetrafluroethylene (Teflon™) can be used for the formation of film. However, plastic petri dishes and boxes of plastic and polytetrafluroethylene (Teflon™) is high in demand due to high inertness and good appearance of the film. After homogenization process, the casting material (film forming solution) was kept undisturbed for 30-45 min in desiccator. This is referred as Ageing & is required for the settlement of bubbles and to decrease the temperature of the film forming solution and further it is transfer to drying oven for its drying.

3.3.2.4 Drying

The heating instrument required for the surface drying can be hot drying oven, incubator, stability chamber etc. Different temperature time combination can be used for the formation of film such as room temperature (25°C) for 48 hours, 30°C for 18-24 hour, 40°C for 16-17 hours, 50°C for 8-9 hours, 60°C for 3-4 Hours. The temperature time combination used for the formation of film was 40°C for 16-17 hours for drying of the film considering requisite & nature of film in hot drying oven (MAC Hot Air Oven, Model no.- #OUP-95SS WP). Fumigation of the hot air oven was done to suppress the growth of unwanted bacteria on the film during drying

process. In the drying step, the solvent used is the preparation of film forming solution was evaporated by heating at controlled temperature at specific time, so that the properties of the formed was not effected by heating.

3.3.3 Wrapping of the edible film on Milk Cake

The milk cake prepared (refer sec. 3.3.1) for wrapping was analyzed for its physico-chemical properties. The pieces of the freshly prepared milk cake were cut according to the size which can be easily consumed by consumer at the time. The pieces of milk cake were cut approx. 4 cm in length, 2 cm in breadth and 1 cm in height. The film prepared for wrapping the milk cake was cut according to the sizes of the pieces of the milk cake. The piece of the milk cake was kept on the flat surface and the film was wrapped on the surface of piece in such a way so that maximum of the piece could be properly wrapped with film, replicating the wrapping of milk cake with butter paper.

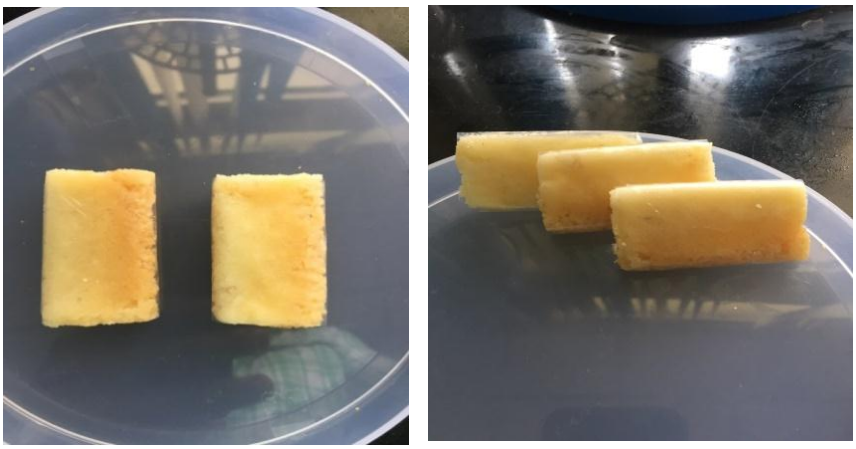


Figure 3.2: Wrapping of milk cake with edible film

3.4 Sensory Evaluation

The sensory evaluation of prepared samples was conducted in College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, with a panel of seven semi trained members. Prior to sensory evaluation, sensory panel was briefed about the desirable characteristics of the product wrapped with edible film. Nine-point hedonic scale was employed to carry out the evaluation of samples (Amerine *et al* 1965 and Shone *et al* 1979) with a view to access the favorable reception of the optimized formulation concerning the sensory attributes of colour and appearance, flavour, mouth feel as well as overall

acceptability, a 9-point structured hedonic scale test (Appendix I) (9 = "extremely like"; 5 = "neither like nor dislike"; 1 = "extremely dislike") was used. The nine-point hedonic scale occupies a unique place in terms of its general applicability to the measurement of product acceptance/preference (Stone and Sidel 2004). However, this method does not reflect actual consumer perception, but it strongly indicates attributes which a good quality product should possess (Arora *et al* 2010).

3.5 Storage Study

Optimized product was stored at ambient temperature (25°C) and refrigeration temperature (4°C) in a refrigerator (make: LG, Model: GL-M472GSHM/2014, Capacity: - 420 litres) for shelf life estimation. Stored product was evaluated for sensory, physico-chemical and microbiological count after every three days till product was safe for consumption, and no abnormal taste was detected. The sensory attributes of optimized product on a 9- point hedonic scale were studied by panel of seven semi-trained judges. Under mechanical testing various attributes such as thickness, tearing strength, puncturing strength, water activity, WVTR, water solubility, transmittance was studied. The microbial attributes like SPC, Coliforms, Yeast and molds were also studied. All the chemical and microbial tests were done in triplicates and distilled water was used for accuracy in the estimation.

3.6 Analytical Procedures

The brief description of different methods used to examine physico-chemical properties, characterization and microbiological studies of edible film is given below.

3.6.1 Physico-chemical analysis

Different standard procedures were adopted to deeply analyze the samples for various physico chemical attributes such as moisture, ash, pH and Titratable acidity were analyzed as per given procedure.

3.6.1.1 Moisture Content of product

The moisture content of milk cake along with film was calculated by moisture analyzer. 2g sample was kept on the drying plate of Mettler Toledo at 105±1ºC, till it show the readings.

3.6.1.2 Ash content measurement

Ash content was determined by adopting the procedure given by AOAC (2006).About 1 g of the sample was weighed accurately in a silica dish and heat on a

Bunsen burner with final incineration in a muffle furnace at 550±5°C for 3 hours. After incineration, it was allowed to cool by transferring the dishes to desiccator. After proper cooling, dishes contained ashed sample was weighed till the difference between the two consecutive weighings was less than 0.2 mg. Ash content was expressed as percent of the gross product.

3.6.1.3 Titratable acidity (% lactic acid)

The titratable acidity was determined as per method of AOAC (1975). Two g of sample was taken in a volumetric flask and mixed homogenously by adding 20 ml hot distilled water (65°C). This was followed by addition of 10 ml of 0.1N sodium hydroxide and 1 ml of 0.5% phenolphthalein indicator. The mixture was titrated against 0.1N hydrochloric acid with continuous stirring till the pink colour disappeared completely. Acidity was expressed as lactic acid/g of sample.

3.6.1.4 pH

Ten g of sample was weighed in a 50 mL beaker and was analyzed by digital pH-analyzer. The pH was measured in triplicates and after each reading the electrode was dipped into distilled water and wiped off.

3.6.2 Storage analysis

3.6.2.1 Total and Reducing sugars

Sugars were determined following the method prescribed by BIS (1981). Accurately weighed 40 g of sample was taken in 100 ml beaker to which 50 ml of hot water (80-90°C) was added and after thorough mixing, contents were transferred to 250 ml volumetric flask. Final volume was made up to about 120-150 ml. The contents in the volumetric flask were then mixed and cooled to room temperature followed by addition of 5 ml of 10% dilute ammonia. After 15 minutes, 5 ml of 10% dilute acetic acid was added to neutralize the added ammonia. To this was added, 12.5 ml zinc acetate solution followed by 12.5 ml of potassium ferrocyanide solution and mixed again. Final content was made to 250 ml volume using distilled water and further filtered through Whatman no. 1 filter paper. The filtrate thus obtained was marked as B_1. From B_1 25 ml was taken into a 100 ml volumetric flask and 5 ml of conc. HCl was added followed by heating at 68°C for 5 minutes. The same was

cooled and neutralized with 50% NaOH and made upto 100 ml with distilled water and was marked as A_1. The solution marked as A_1 was diluted 20 times while B_1 was diluted 4 times and were marked as A_2 and B_2 respectively. Both the solutions were taken into a burette and titrated against the mixture of 5 ml each of Fehling A and Fehling B solutions added with a mixed indicator. Similarly, standard lactose and sucrose were taken and titrated. For calculation, the following formula was used:

10 ml of Fehling (A+B) solution = V_1 ml of standard invert sugar solution of concentration C_1 mg/L (i.e. C_1=2.5 mg/ml)

10 ml of Fehling (A+B) solution = V_2 ml of sample solution before inversion of concentration C_2 mg/ml

10 ml of Fehling (A+B) solution = V_3 ml of inverted sample filtrate having concentration C_3 mg/ml

$$V_1C_1 = V_2C_2$$

$$\therefore C_2 = \frac{V_1C_1}{V_2}\frac{mg}{mL}$$

$$C_2 = \frac{V_1C_1}{V_2 * 1000} * \frac{250}{40} * \frac{100}{25} * 100\ \%$$

$$C_3 = \frac{V_1C_1}{V_3 * 1000} * \frac{250}{40} * \frac{100}{25} * \frac{100}{25} * 100\ \%$$

3.6.2.2 Free fatty acid

The method prescribed by Deeth *et al* (1975) was used to estimate the FFA content of sample. The method consisted of accurate weighing of 5g of sample into a 60 ml stoppered test tube. Ten ml of extraction mixture (Isopropanol: petroleum ether: 4N H_2SO_4 in the ratio of (40:10:1) was added and mixed thoroughly. This was followed by the addition of 6 ml petroleum ether and 4 ml distilled water. The test tube was stoppered and tempered at 40°C for 10 minutes the contents were vigorously shaken for 20 sec. The two layers were allowed to separate for 10-15 minutes and an aliquot of the upper layer (5-8 ml) was withdrawn and titrated against 0.02 N methanolic KOH solution using 1% methanolic phenolphthalein indicator. The FFA content of sample was obtained from the following formula:

$$\text{FFA } (\mu \text{ eq/g}) = \frac{TN}{PW} \times 1000$$

Where,

T= ml of 0.02 N KOH used

N = Normality of methanolic KOH solution

P = Proportion of upper layer of aliquot titrated / total aliquot

W = Weight of sample (g)

3.6.2.3 Tyrosine value

Tyrosine value can be used to check the level of proteolysis in foods. Tyrosine value is useful as a general index of bacteriological and organoleptic quality of commercial raw milk subjected to prolonged cold storage.

Five grams of sample was exactly weighed and taken in a clean dry test tube, added with 10 ml of distilled water followed by addition of 10 ml of 0.72 N TCA (117.64 g TCA 1000 ml^{-1} solution). The test tubes were then stoppered shaken vigorously and incubated at 27°C for 10 minutes. The precipitated proteins were filtered through Whatman No. 42 filter paper and the tyrosine content in the filtrate was determined. To 5.0 ml of this TCA soluble filtrate, 2.0 ml of distilled water, 10 ml of sodium carbonate reagent (75.0 g anhydrous Na_2CO_3 and 10.0 g sodium tetra phosphate dissolved in glass distilled water and diluted to 500 ml) followed by 3.0 ml of the Folin's phenol reagent (Folin's phenol reagent diluted 1: 2 with distilled water) was added, mixed well and incubated again at 27°C for 10 minutes for colour development. The intensity of blue colour so developed was measured at 650 nm using spectrophotometer.

The following regression equation given by Chawla (2010) was used to calculate the tyrosine concentration (μg 5 ml^{-1}).

$$Y = 0.1024X + 0.1197$$

Where,

X = Concentration of tyrosine in μg 5 ml^{-1}

Y = absorbance at the respective tyrosine concentration.

3.6.2.4 Hydroxy methyl furfural (HMF)

The HMF value is an indicator of the freshness and storage period of a product. Hydroxy methyl furfural (HMF) is an organic compound derived from dehydration of certain sugars. This yellow low-melting solid is highly water-soluble. HMF is practically not present in fresh food, but it is naturally generated in sugar-containing

food during heat-treatments like drying or cooking. Along with many other flavour- and colour-related substances, HMF is formed in the Maillard reaction as well as during caramelization. In these foods it is also slowly generated during storage. Total HMF in samples (stored) was determined by the method recommended by Keeney and Bassette (1959) with slight modification.

Three g of sample was thoroughly mixed with 7 ml distilled water. Then 5 ml of 0.3N oxalic acid was added and the tube was kept in boiling water bath for 60 minutes. The contents of the tube were cooled and subsequently 5 ml of 40% trichloroacetic acid solution was added and precipitation occurred. The precipitated mixture was filtered through Whatman No. 42 filter paper. 0.5 ml of the filtrate was pipetted out into a 5 ml test tube and added with 3.5 ml of distilled water and 1 ml of 0.05M Thiobarbituric acid solution (aq) and mixed well. The tubes were kept in water bath at 40°C for 50 minutes. After cooling to room temperature, absorbance was measured at 443 nm. A blank test was carried out in the same manner as above substituting distilled water for sample

Total HMF (μ mol/100mg) = (Absorbance – 0.055) x 87.5 x 0.4

3.6.2.5 Thio barbituric Acid (TBA) Value

The extent of oxidation of fat in sample was measured in terms of TBA Value. The extraction method of Strange *et al* (1977) was followed with slight modification. For TBA value determination, about 2 g sample was taken and blended with 50 ml of 20% TCA (Tri-chloroacetic acid) and 50 ml of distilled water and the mixture was left undisturbed for 10 minutes. Then the contents were filtered through Whatman No.1 filter paper. The filtrate (5 ml) was pipetted out in clean and dry test tube and added with 5 ml of 0.0l M 2Thiobarbituric acid. Colour was developed by incubating the tubes in boiling water bath for 30 minutes. The contents were cooled to room temperature and absorbance was recorded at 532 nm. Blank determinations were made using distilled water in place of sample. TBA value was expressed as absorbance (O.D.) at 532 nm.

3.6.2.6 Peroxide Value

The peroxide value of Milk cake was determined by the procedure as described in AOAC (1970). Fat was first extracted from 5 g of sample soaked in 30

ml chloroform for 12 hours in an air tight separating funnel. The chloroform extract was transferred to 60 ml tube. 1 g of potassium iodide and 10 ml acetic acid were added. The tube was kept in a boiling water-bath for 30 seconds. The tube was then cooled immediately in tap water. In a conical flask, 20 ml of 5 % (w/v) potassium iodide solution was taken and to it the content of the above tube were transferred with washing with 20 ml distilled water. The contents of conical flask were then titrated against 0.002 normal sodium thiosulphate solution using 2 ml of 1 % starch indicator. A blank was also run simultaneously without sample. The peroxide value was expressed as meq of O_2/kg fat of sample.

3.6.2 Characterization of film

3.6.2.1 Film Thickness

The thickness of the film was determined with a digital micrometer (Mitutoyo Digimatic Micrometer, No. 293-831-30) with an accuracy of 0.001 mm. Twelve measurements were taken at different points randomly selected from each film and an average was obtained. Also average thickness value was measured by folding the film and measured from different points. Thickness of the film carries a significant importance as variation in the thickness affect the different properties such water vapor transmission rate, water activity, permeability of various gases etc.

3.6.2.2 Moisture Content of film

The moisture content (MC) of films was measured by the gravimetric method. For this, 500 mg of film was dried in air circulating oven at for 24 h (till the equilibrium weight was attained). The removal of moisture was determined and moisture content was calculated as percentage of water removed from the film.

3.6.2.3 Water Solubility

The water solubility of films was determined as the content of dry matter solubilized after 24 h of immersion in water. 500 mg film sample was immersed in 50 ml of water at 25± 1 °C) for 24 h of immersion with agitation at regular interval. After that undissolved part of film were taken out and dried till constant weight achieved in a hot air oven at 105±1°C. Solubility (%) was calculated as:

$$\text{Solubility (\%)} = \frac{(\text{Wt. of initial dry film} - \text{Wt. of un dissolved film})}{\text{Wt. of initial dry film}} \times 100$$

3.6.2.4 Water Vapor Transmission Rate (WVTR)

Water vapor transmission rate was measured using a modified ASTM 96–00 method (ASTM 2000). The film was sealed on a modified test cell (beaker) containing 30 mL of distilled water. The test cell was then kept in a desiccator containing pre-dehydrated silica gel. Silica gels were dried at 180 °C for 3 h for these measurements. The whole assembly was kept at 25 °C and weight loss of the test cell was measured after storage for 24h. WVTR of the film was calculated according to the equation,

$$WVTR=\Delta W/(\Delta t\times A)$$

where ΔW is the weight loss of test cell, Δt is the time of storage, and A is the area of exposed film.

3.6.2.5 Transmittance

Transmittance of films was determined by placing the film strips (3 cm×1 cm) in the cuvette containing water and was measured the percentage transmittance at fixed wavelength of 660 nm using UV–VIS spectrophotometer (Systronics Double Beam Spectrophotometer 2203 ^{smart}).

3.6.2.6 Tearing strength

Tearing strength of films was measured by placing 6-7 cm diameter film strip in between two grips or clumps. Distance between two grips was 5 cm and speed of the instrument was 1mm/s. The minimum force required to rupture the film was calculated in N (Newton). Each test film requires at least 3 replicate measurement. Tensile strength was measured in TMS- Pro Food Technology Corporation texture analyzer.

3.6.2.7 Puncturing Strength

Puncturing strength of films was measured by placing 6-7 cm diameter round film strip on a circular holder. Speed of the instrument was 1mm/s. The minimum force required to pierced the film was calculated in N (Newton). Each test film requires atleast 3 replicate measurement. Puncturing strength was measured in TMS-Pro Food Technology Corporation texture analyser.

3.6.2.8 Water activity

The water activity of film was determined using water activity meter (Aqua Lab, Series 4TE) and was previously calibrated with water at 25^{o}C. The average of three measurements were taken as result.

3.6.2.9 Instrumental Colour measurement

Surface color of edible film samples were measured using Color Flex Colorimeter (Hunterlab, Reston, Virginia) supplied along with the universal software Easy Match QC (version 4.62) and the results were expressed in terms of CIE-LAB system. The colorimeter was equipped with dual beam xenon flash lamp as source of light. Prior to analyzing the samples, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer of the equipment. Data was received through the software in terms of L* (lightness), ranging from 0 (black) to 100 (white), a* (redness), ranging from +60 (red) to -60 (green) and b* (yellowness), ranging from +60 (yellow) to -60 (blue).

3.6.2.10 Structural integrity

Microstructure of film samples of the film surface was observed using Scanning electron microscope (Hitachi s-3400N, Japan) at accelerating voltage of 15 kV. A small of piece of each film was placed on a stub using two sided carbon tape and then sputter coated with a thin layer of gold prior to SEM observations. The surface of film samples were obtained during testing at ambient temperature using initial grip distance of 100mm and crosshead speed of 50mm/min. The micrographs representing the ultrastructure of the edible film were taken by instrument`s software installed on a PC connected to the system.

3.6.3 Microbiological Studies

Standard plate count, yeast and mold count and coliform count were recorded as per procedure by Wehr and Frank (2004) using the media Nutrient agar for standard plate count, Potato dextrose agar for yeast and mold and MacConkey broth for coliform.

3.6.3.1Preparation of dilution blanks

The dilution blank consisted of 0.08% (w/v) sterile sodium chloride solution (AR grade, procured from Central Drug House) in 99 ml and 9 ml portions in screw

capped dilution test tubes. These were autoclaved at a pressure (121°C) for 20 minutes. The dilution blanks were warmed to 45°C before use for preparation of samples.

3.6.3.2 Preparations of sample

One gram of film sample was weighed in sterile aluminium dish, in the balance. The contents of the aluminium dish were then transferred to a sterile Pestle morter and get crashed with 9 ml of dilution sample. This gives a dilution of 1:10 from this initial dilution, further dilutions were prepared by transferring 1 ml into 9 ml blanks.

3.6.3.3 Standard plate count

Nutrient agar was used to enumerate the standard plate counts in the starch based edible film.

Media was rehydrated by dissolving 28 g of dry media in 1000 ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes.

0.1 ml of the diluted sample (suitable dilution, 10^{-1}, 10^{-2}, 10^{-3}) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and 0anti-clockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 37°C for 24-48 hrs.

3.6.3.4 Yeast and mould count

The count was enumerated using Potato dextrose agar (PDA). Media was rehydrated by dissolving 44 g of dry media in 1000 ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes. One ml of 10% tartaric acid was added to lower the pH of the media to 3.5.

0.1ml of the diluted sample (suitable dilution, 10^{-1}, 10^{-2}, 10^{-3}) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted

agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then incubated at 25±2°C for 3 to 4 days.

3.6.3.5 Coliform count

The count was enumerated using MacConkey broth. Media was rehydrated by dissolving 34.51g of MacConkey broth and 15g of agar powder in 1000 ml distilled water. The mixture was then boiled to dissolve the medium completely and then cooled to 45°C. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes.

0.1 ml of the diluted sample (suitable dilution, 10^{-1}, 10^{-2}, 10^{-3}) was transferred in each of the triplicate petri dishes. Then in each Petri dish 15-20 ml of the melted agar (at 45°C) was poured, and the contents were mixed well by rotating in a clock wise and anti-clockwise position slowly. The contents were allowed to solidify at room temperature then a second layer of agar was made by adding 5-10 mL of melted agar. The media was allowed to solidify and then incubated after inverting the plates.

3.7 Consumer Study

To know the acceptability of developed product, final optimized sample of milk cake wrapped with edible packaging was offered to 110 respondents considering different age groups, profession and custom & traditions to find acceptability of beverage (Alvensleben and Schrader1998). Consumer's response about the product was recorded through questionnaire (Appendix II) supplied along with the samples to all consumers participated in consumer survey.

3.8 Statistical Analysis

The data obtained during the present investigation was analyzed by employing mention statistical designs. The quality of milk cake with and without edible film in sensory, physico-chemical and microbiological terms during storage study of the product was assessed by employing one way analysis of variance (ANOVA) and Turkey's multiple range tests using IBM SPSS statistics version 20. The result were compiled in Microsoft excel (Microsoft office 2016). All the data collected was expressed as mean ± S.D from three independent samples.

CHAPTER IV

RESULT AND DISCUSSION

The investigation entitled “Development and Characterization of edible film for packaging of milk cake” was undertaken to replace the conventional packaging material i.e. butter paper, considering a load from environment point of view. The study was conducted in three phases. The first phase of study was to conduct preliminary trials for getting a broader range of variables (raw material for edible film preparation) under consideration to be optimized. Second phase consisted of narrowing down of variables by analyzing the properties of the film. In third phase, storage study was conducted for evaluating its shelf life in terms of sensory, physico-chemical and microbiological changes. The later part of the study is related with consumer response studies of the developed product. The results obtained during these studies are presented and discussed in detail herein this chapter.

4.1 Screening of various polymers and plasticizers

Starch is widely used as the base component for making edible film because it has very good film forming properties. Starch contains amylopectin and amylose contents, to varying range which are helpful for making the compact structure of the film. The film forming properties of any polysaccharide depend upon the amylose amylopectin ratio of the starch. Also, food grade biopolymers give brittle and stiff characteristics due to excessive interactions between the polymer molecules. Various sources of starches such as wheat, corn and rice were tried in this study. Different preliminary trials were conducted with broader range of different starches and plasticizers to study the detailed overview of these variables. To build up the physical and mechanical properties & to increase the flexibility and tearing strength, different polyols such as glycerol, mannitol and sorbitol were analyzed as a plasticizer.

Plasticizer is an essentially required raw material helpful in providing desired flexibility to the film. Different trials were undertaken at specific temperature and time conditions. The results of these trials have been depicted in the Table 4.1.

Table 4.1: Development of film with different concentrations of various starches and proteins with glycerol as a plasticizer

Type of starch	Plasticizer (Glycerol)	Observations for film formation
Corn starch 4%	2%	Torn Off
Corn starch 5%	**2%**	**Very Good Formation**
Corn starch 6%	2%	Difficult to Peel
Corn starch 4%	3%	Hard Formation
Corn starch 5%	3%	Wrinkled
Corn starch 6%	3%	Not Properly Formed
Wheat starch 4%	2%	Hard Formation
Wheat starch 5%	**2%**	**Good formation**
Wheat starch 6%	2%	Wrinkled
Wheat starch 4%	3%	Torn Off
Wheat starch 5%	3%	No Formation
Wheat starch 6%	3%	Difficult to Peel
Rice starch 4%	2%	Torn Off
Rice starch 5%	**2%**	**Good formation**
Rice starch 6%	2%	Difficult to Peel
Rice starch 4%	3%	Hard Formation
Rice starch 5%	3%	Wrinkled
Rice starch 6%	3%	Difficult to Peel
Sesame protein isolate (4%)	2%	Wrinkled
Sesame protein isolate (5%)	2%	Torn off
Sesame protein isolate (6%)	2%	Off smell
Sesame protein isolate (4%)	3%	Dark color
Sesame protein isolate (5%)	3%	Wrinkled
Sesame protein isolate (6%)	3%	Off smell
Soy protein isolate (4%)	2%	Torn off
Soy protein isolate (5%)	2%	Off smell
Soy protein isolate (6%)	**2%**	**Good formation**
Soy protein isolate (4%)	3%	Dark color
Soy protein isolate (5%)	3%	Off smell
Soy protein isolate (6%)	3%	Torn off

Table 4.2: Development of film with different concentrations of various starches and proteins with mannitol as a plasticizer

Type of starch	Plasticizer (Mannitol)	Observations for film formation
Corn starch 4%	2%	Not Properly Formed
Corn starch 5%	2%	Wrinkled
Corn starch 6%	2%	Torn Off
Corn starch 4%	3%	Hard Formation
Corn starch 5%	3%	Wrinkled
Corn starch 6%	3%	Difficult to Peel
Wheat starch 4%	2%	Torn Off
Wheat starch 5%	**2%**	**Good formation**
Wheat starch 6%	2%	Wrinkled
Wheat starch 4%	3%	Torn Off
Wheat starch 5%	3%	Not Properly Formed
Wheat starch 6%	3%	Difficult to Peel
Rice starch 4%	2%	Torn Off
Rice starch 5%	2%	Wrinkled
Rice starch 6%	2%	Difficult to Peel
Rice starch 4%	3%	Hard Formation
Rice starch 5%	3%	Wrinkled
Rice starch 6%	3%	Torn Off
Sesame protein isolate (4%)	2%	Torn off
Sesame protein isolate (5%)	2%	Wrinkled
Sesame protein isolate (6%)	2%	Off smell
Sesame protein isolate (4%)	3%	Torn off
Sesame protein isolate (5%)	3%	Dark color
Sesame protein isolate (6%)	3%	Wrinkled
Soy protein isolate (4%)	2%	Off smell
Soy protein isolate (5%)	2%	Torn off
Soy protein isolate (6%)	2%	Off smell
Soy protein isolate (4%)	3%	Torn off
Soy protein isolate (5%)	3%	Dark color
Soy protein isolate (6%)	3%	Difficult to peel

Table 4.3: Development of film with different concentrations of various starches and proteins with sorbitol as a plasticizer

Type of starch	Plasticizer (Sorbitol)	Observations for film formation
Corn starch 4%	2%	Wrinkled
Corn starch 5%	2%	Torn Off
Corn starch 6%	2%	Not Properly Formed
Corn starch 4%	3%	Hard Formation
Corn starch 5%	3%	Wrinkled
Corn starch 6%	3%	Difficult to Peel
Wheat starch 4%	2%	Torn Off
Wheat starch 5%	2%	Not Properly Formed
Wheat starch 6%	2%	Difficult to Peel
Wheat starch 4%	3%	Wrinkled
Wheat starch 5%	3%	Torn Off
Wheat starch 6%	3%	Not Properly Formed
Rice starch 4%	2%	Difficult to Peel
Rice starch 5%	2%	Hard Formation
Rice starch 6%	2%	Torn Off
Rice starch 4%	3%	Wrinkled
Rice starch 5%	3%	Hard formation
Rice starch 6%	3%	Difficult to Peel
Sesame protein isolate (4%)	2%	Off smell
Sesame protein isolate (5%)	2%	Torn off
Sesame protein isolate (6%)	2%	Dark color
Sesame protein isolate (4%)	3%	Torn off
Sesame protein isolate (5%)	3%	Dark color
Sesame protein isolate (6%)	3%	Wrinkled
Soy protein isolate (4%)	2%	Off smell
Soy protein isolate (5%)	2%	Wrinkled
Soy protein isolate (6%)	2%	Off smell
Soy protein isolate (4%)	3%	Torn off
Soy protein isolate (5%)	**3%**	**Good formation but off smell**
Soy protein isolate (6%)	3%	Wrinkled

It is evident from the Table 4.1, starch varied from 4-6 per cent, along with glycerol as a plasticizer at the level of 2-3 per cent. It can be concluded from the Table 4.1, that corn starch when added at a concentration of 5 per cent along with 2 per cent glycerol, was able to give film with desired visual & physico-mechanical properties. This film was easy to peel off without any fear of getting torn off. However, film with lesser or higher concentration of starch was either torn off or was sticked to the base of petri plate while removing the same when used @ as 4 or 6 per cent, respectively. Similarly, even wheat and rice starch showed the somewhat similar results. Lahohakunjit *et al* 2004 prepared rice starch film and investigated the effect of plasticizers, glycerol, sorbitol and polyethylene glycol 400 (PEG_{400}), on mechanical and barrier properties of rice starch film. Also, protein-based polymers such as soy protein isolate at 6 per cent concentration gave good formation with 2 per cent concentration of glycerol. Similar study was conducted by Soliman *et al* 2007 wherein authors described the formation of film using soy protein isolate and polyethylene glycol (PEG_{400}) at a concentration of 60% as a plasticizer, which led to the better mechanical and barrier properties. Reference study Table 4.2 and 4.3 depicts use of similar concentration of starches replacing glycerol with mannitol or sorbitol to evaluate their performance as a plasticizer. Mannitol could only give a good quality film with wheat starch at 2 per cent concentration whereas with other starches at different concentration mannitol could not provide required plasticization effect. Similar study was undertaken by Farahnaky *et al* 2013 describe the effect of glycerol on properties of wheat starch edible film. Sorbitol at different concentration was also unable to give required flexibility and peeling off character to the films when used with varying concentration of different starches. It gave good formation but had bit off smell in case of soy protein isolate at 5 percent concentration with 3 per cent concentration of sorbitol (Table 4.3), when compared with corn starch films, prepared earlier in this study.

Amongst various starches, though rice film was more transparent and whiter in colour but comparing the different physico-chemical properties, corn starch along with glycerol at 5 and 2 per cent concentrations, respectively were evaluated as best variables for edible film preparation bearing required transparency, strength and good visual appearance (Figure 4.1). Various physico-chemical and microbiological

properties of corn, wheat and rice starch (@5%) and glycerol (@2%) were evaluated for their further efficacy (Table 4.4 and 4.5), as a material of the film formation.

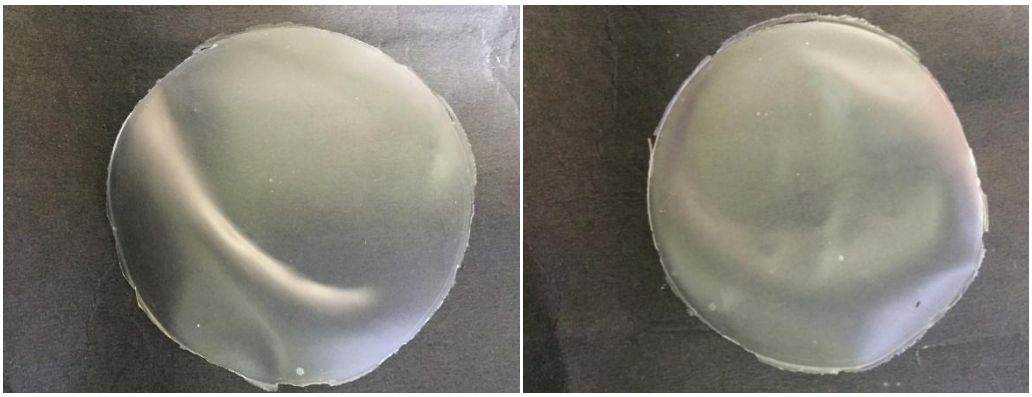


Figure 4.1: Film prepared by corn starch & Glycerol @5 and 2%, respectively

Table 4.4: Physico-chemical and mechanical properties of edible film prepared from different starches (Corn, Wheat & Rice)

Properties	Corn starch (5%)	Wheat starch (5%)	Rice starch (5%)
Thickness (mm)	0.0813 ± 0.05^{b}	0.0934 ± 0.03^{a}	0.095 ± 0.06^{a}
WVTR (g/m^2h)	7.38 ± 0.13^{b}	7.68 ± 0.09^{a}	7.64 ± 0.17^{a}
Water solubility (%)	26.20 ± 1.15^{b}	25.47 ± 0.97^{a}	24.12 ± 1.07^{a}
Moisture (%)	22.30 ± 1.43^{b}	23.12 ± 1.61^{a}	23.04 ± 1.43^{a}
Tensile strength (N)	8.65 ± 1.14^{a}	8.40 ± 1.03^{a}	7.31 ± 1.17^{b}
Puncturing strength (N)	2.16 ± 0.83^{a}	2.09 ± 0.77^{b}	1.98 ± 0.95^{c}
Color	L = 32.06 ± 0.27^{a} a = -0.16 ± 0.02^{c} b = -0.28 ± 0.23^{c}	L = 26.27 ± 0.43^{b} a = -0.09 ± 0.03^{b} b = -0.43 ± 0.17^{b}	L = 9.20 ± 0.19^{c} a = -0.03 ± 0.01^{a} b = -0.17 ± 0.09^{a}
Water activity	0.475 ± 0.02^{a}	0.472 ± 0.03^{a}	0.474 ± 0.02^{a}
Transmittance (%)	73.40 ± 1.5^{a}	66.20 ± 2.3^{b}	74.20 ± 1.7^{a}

Values are the average of triplicates ± standard deviation

Table 4.4 shows the various mechanical properties of film prepared using different starches of 5 per cent concentration along with 2 per cent glycerol. It was

observed that, the film prepared by corn starch showed less thickness, WVTR, & per cent moisture content than the film prepared from the other starches, which are desirable characteristics for making good edible film. The results also showed the good mechanical properties such as tensile strength and puncturing strength which were also prominently recognizable in corn starch film as the desirable properties. Thus, corn starch showed significant difference ($p<0.05$) with other starches. From these observations, it was found that the corn starch is best suited among all other starches when used at 5 per cent concentration.

Table 4.5: Microbiological properties of edible film prepared from different starches (Corn, Wheat & Rice)

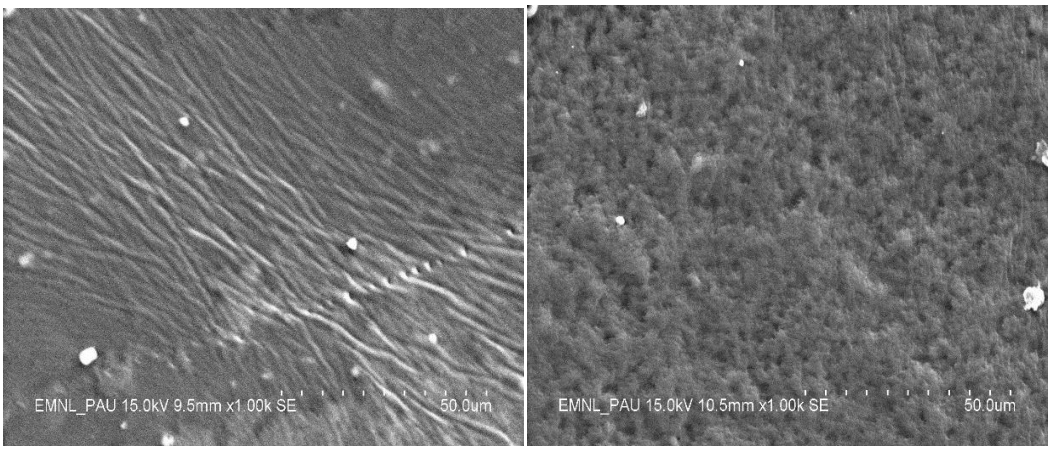
Count ($\log_{10}$cfu/g)	**Corn (5%)**	**Wheat (5%)**	**Rice (5%)**
SPC	3.78 ± 0.18^{b}	4.04 ± 0.35^{a}	3.78 ± 0.18^{b}
Coliform	NIL	NIL	NIL
Yeast and Mold	NIL	NIL	NIL

Values are the average of triplicates ± standard deviation

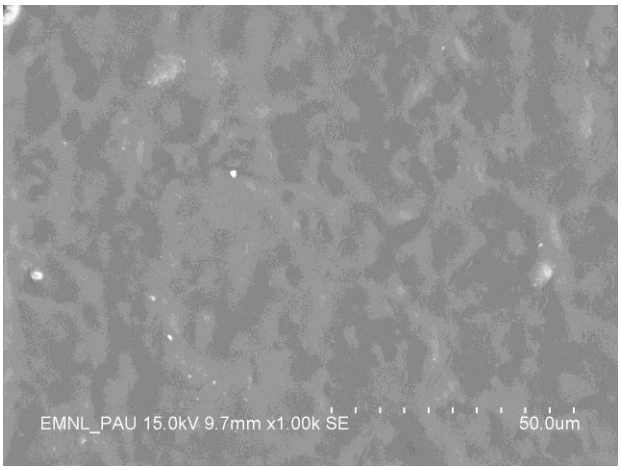
Also, from the microbial point of view, SPC, coliform and yeast & molds of film prepared from three different starches (Corn, Wheat & Rice) were analyzed for the microbial count (Table 4.5). There was similar count of SPC for the film prepared from corn starch and rice starch, whereas the film prepared by wheat starch showed high SPC. There was no coliform and yeast & mold growth noticed in all these films. Also, Permissible limits of FSSAI for khoa or khoa based sweets are far more than the observed values (SPC not more than 50000/g) Hence, conforming that packaging can be used as a medium of wrapping without fear of introducing growth to the product. Thus, according to the results obtained, corn starch film showed low SPC, nil colonies of coliform and yeast & mold growth, as compared to the film prepared from the other starches (Table 4.5).

Structural Integrity: Structural integrity of the films prepared from these three starches were also analyzed to examine the structural morphology of the films (Figure 4.2). Depending on the nature of the components and the interactions occurring in the film-forming suspensions, the hydrocolloids arrange themselves in different ways in the

film matrices. Cross-sections of all starch films display heterogeneities, looking like a network of fibers. However, according to the very homogeneous and smooth aspect of



Rice starch **Wheat Starch**



Corn Starch

Figure 4.2: Scanning Electron Microscopy of the film prepared from corn, wheat & Rice starch, at 1.00k

the surface, there was no porosity. This microstructure could be affected by incomplete dissolution/ gelatinization of starch granules linked by the solubilized-gelatinized starch fraction. The film prepared from the rice starch showed fiber like structure, which led to more porosity of film. On the other hand, film prepared from corn and wheat starch showed less heterogeneous structure than rice starch film. Therefore, it improved the physico-chemical and mechanical properties of film. According to Basiak (2017) the heterogeneity of the film depends upon the amylose/ amylopectin ratio. The film having low amylose content leads to decrease in the thickness of the film and have homogenous structure. The more dense structure of the

film with less thickness was observed in films prepared from corn starch (amylose: amylopectin was 20:80).

4.2 Physico-chemical properties of optimized film

From various results obtained in the study, it can be concluded that corn starch & glycerol if used @ 5 & 2% respectively, can yield a film with desired properties. This film showed good physico-chemical & microbiological properties than other starches as shown in Table 4.4 & 4.5. Thus, can be taken as an optimized ratio for film formation. Different physico-chemical & microbiological properties were analyzed for the optimized film and have been depicted in Table 4.6.

4.3 Shelf life evaluation of optimized product with edible film

As we know microbial contamination is one of the main sources of spoilage of food goods. Also, with the passage of time food in general, leads to the loss of quality due to the physical, chemical & microbiological changes. These changes depend upon the composition, moisture, processing or techniques of packaging. These deteriorative changes can be observed in the storage which will decide the shelf life of the product. Also, during storage, there are several decomposition and formation process occur, which leads to the chemical and biochemical changes which make the product less acceptable during the storage. Conventionally butter paper around the sweets was formulated to enhance the shelf life of the product. To eliminate the use of paper & introduce a means to serve in package product, edible film products were designed. Though the product with edible films contains little moisture content, which led to little change in physico-chemical and biochemical changes during storage. But again, the role of temperature cannot be ignored, as the extent of deterioration is further influenced by the keeping temperature i.e. temperature during storage and storage period i.e. time. These changes are of much significance because they affect the overall acceptability. This section of the present investigation deals with the assessment of changes in microbial, physico-chemical, and sensory quality of milk cake with or without edible film during ambient and refrigerated storage at 25 and 4°C respectively.

Table 4.6: Different physico chemical & mechanical properties of optimized film (on 0 day)

Sr. No.	Parameter	Value
Physico chemical & mechanical properties of film (mean±SD)		
1.	Thickness (mm)	0.0832±0.04
2.	Moisture (%)	22.40 ±1.21
3.	WVTR (g/m^2h)	7.44± 0.15
4.	Water solubility (%)	25.90±1.09
5.	Water activity	0.477±0.07
6.	Puncturing Strength (N)	2.17±0.79
7.	Tearing strength (N)	8.59±0.12
8.	Transmittance (%)	72.60±2.1
Instrumental Colour Measurement		
8.	L*	31.56 ± 0.22
9.	a *	-0.14 ± 0.02
10.	b*	-0.29 ± 0.03
Microbiological count		
15.	SPC ($\log_{10}$cfu/g)	3.79±0.35
16.	Yeast & mold (($\log_{10}$cfu/g)	NIL
17.	Coliform (($\log_{10}$cfu/g)	NIL

Values are the average of triplicates ± standard deviation

For shelf life evaluation, the product milk cake was wrapped in edible films to a maximum extent of hygienic condition to avoid contamination and further kept at ambient (25 ± 1°C) and refrigeration (4°C) temperature, till spoilage. The product was analyzed for its sensory attributes, physico-chemical properties and microbiological parameters at the regular interval of 3 days at both the temperatures till spoilage. Sensory profile of stored product was evaluated by a panel of seven semi-trained panelists on nine point-hedonic scale as elaborated wide section 3.5. Physico-chemical, and microbiological parameters were analyzed as per the procedure mentioned in the section 3.8.1 and 3.8.3, respectively.

4.3.1 Effect of storage on sensory properties of milk cake with edible film

The sensory scores of milk cake with edible film decreased gradually during storage at both refrigeration (4°C) and ambient (25°C) temperature because of continuous decrease in moisture content with the progression of storage period. All the sensory parameters decreased slowly at refrigeration temperature as compared to ambient temperature. The color, body & texture, sweetness, flavor and overall acceptability scores of fresh milk cake with edible film (treated) and without edible film (control) at 0 day, when kept at refrigeration temperature (4°C) were 8.00, 7.87, 8.20, 7.86 & 7.75 and 7.75, 7.50, 8.16, 7.80 & 7.80 respectively, which gradually declined during storage at ambient (25°C) and refrigeration temperature (4°C) conditions, as shown in figure 4.3-4.7.

The color scores of control sample decreased from 7.75 to 6.43 after 6 days of storage at ambient temperature (25°C) and sample with edible film decreased from an initial value 8.00 to 7.07 after 6 days of storage at ambient temperature (25°C) while in case of (Table 4.7 & Figure 4.3). On the other hand, the color score of control sample decreased from 7.75 to 5.80 after 18 days of storage at refrigeration temperature (4°C) and score of sample with edible film decreased from an initial value 8.00 to 5.88 after 18 days of storage (Table 4.8 & Fig. 4.3). Color and appearance of the product became light and dull due to dry appearance, as a result of loss of moisture from the upper surface of the milk cake. The decrease in color scores of both control as well as wrapped was statistically significant ($p<0.05$) during storage period. However, color and appearance for sample stored at ambient temperature decreased significantly compared to refrigerated sample after 18 days of storage. Moreover, in the present study, evaporation of moisture during storage might have aggravated the dull appearance of the milk cake as presence of moisture enlivens the appearance of the product by reflecting incident light. Similar effect was experienced by Chatli *et al* 2014 in raw chevon chunk and reported that colour and appearance decreased more rapidly in control than bio-packaged chunks during storage. This may be due to lower water vapour transmission rate of the films, which inhibited surface dehydration and loss of moisture from the product stored at higher temperatures experience.

Table 4.7: Effect of storage on sensory properties of milk cake with edible film at ambient temperature (25°C)

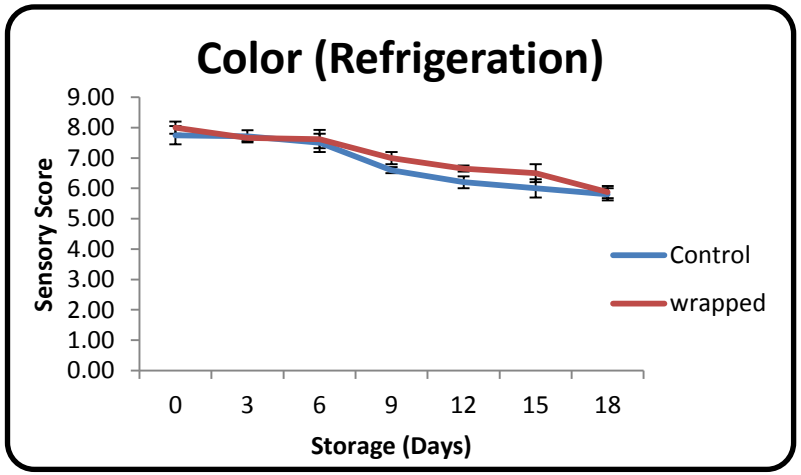
Properties	Sample	Days		
		0	3	6
Color	Control	7.75 ± 0.1^{Ba}	7.66 ± 0.2^{Aa}	6.43 ± 0.1^{Bb}
	Treated	8.00 ± 0.2^{Aa}	7.80 ± 0.3^{Aa}	7.07 ± 0.2^{Ab}
Body & Texture	Control	7.50 ± 0.2^{Aa}	7.40 ± 0.3^{Ab}	6.92 ± 0.2^{Ac}
	Treated	7.87 ± 0.3^{Aa}	7.66 ± 0.2^{Aa}	6.98 ± 0.1^{Ab}
Sweetness	Control	8.16 ± 0.2^{Aa}	7.60 ± 0.1^{Ab}	7.21 ± 0.2^{Ac}
	Treated	8.20 ± 0.3^{Aa}	8.00 ± 0.2^{Aa}	7.30 ± 0.1^{Ab}
Flavor	Control	7.80 ± 0.2^{Aa}	7.62 ± 0.3^{Aa}	6.52 ± 0.4^{Bb}
	Treated	7.86 ± 0.3^{Aa}	7.75 ± 0.4^{Aa}	6.95 ± 0.2^{Ab}
Overall Acceptability	Control	7.80 ± 0.3^{Aa}	7.70 ± 0.4^{Aa}	7.58 ± 0.3^{Aa}
	Treated	7.75 ± 0.4^{Aa}	7.71 ± 0.2^{Aab}	7.67 ± 0.3^{Ab}

Superscript a,b,c shows the significant difference ($p<0.05$) in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs. wrapped product. (N=6),

Table 4.8: Effect of storage on sensory properties of milk cake with edible film at refrigeration temperature (4°C)

Properties	Samples	Days						
		0	3	6	9	12	15	18
Color	Control	7.75 ± 0.3^{Aa}	7.71 ± 0.2^{Aab}	7.50 ± 0.3^{Ab}	6.60 ± 0.1^{Bc}	6.20 ± 0.2^{Bd}	6.00 ± 0.3^{Bde}	5.80 ± 0.2^{Ae}
	Treatment	8.00 ± 0.2^{Aa}	7.66 ± 0.1^{Ab}	7.62 ± 0.3^{Ab}	7.00 ± 0.2^{Ac}	6.65 ± 0.1^{Ad}	6.50 ± 0.3^{Ad}	5.88 ± 0.2^{Ae}
Body & Texture	Control	7.50 ± 0.2^{Ba}	7.47 ± 0.3^{Aa}	6.88 ± 0.1^{Ab}	6.50 ± 0.3^{Ac}	6.30 ± 0.2^{Bcd}	6.10 ± 0.3^{Bd}	6.00 ± 0.1^{Bd}
	Treatment	7.87 ± 0.3^{Aa}	7.66 ± 0.1^{Ab}	6.80 ± 0.2^{Ac}	6.65 ± 0.3^{Acd}	6.53 ± 0.1^{Ade}	6.45 ± 0.2^{Ae}	6.20 ± 0.3^{Af}
Sweetness	Control	8.16 ± 0.3^{Aa}	7.77 ± 0.2^{Ab}	7.33 ± 0.1^{Ac}	7.02 ± 0.2^{Ad}	6.70 ± 0.3^{Ae}	6.50 ± 0.1^{Bef}	6.40 ± 0.2^{Af}
	Treatment	8.20 ± 0.2^{Aa}	7.94 ± 0.1^{Ab}	7.50 ± 0.3^{Ac}	7.13 ± 0.1^{Ad}	6.90 ± 0.2^{Ae}	6.73 ± 0.3^{Aef}	6.60 ± 0.2^{Af}
Flavor	Control	7.80 ± 0.3^{Aa}	7.53 ± 0.2^{Ab}	7.25 ± 0.4^{Ac}	6.75 ± 0.1^{Ad}	6.64 ± 0.2^{Ade}	6.20 ± 0.3^{Ae}	6.00 ± 0.2^{Ae}
	Treatment	7.86 ± 0.3^{Aa}	7.68 ± 0.4^{Aa}	7.42 ± 0.3^{Ab}	6.95 ± 0.1^{Ac}	6.78 ± 0.3^{Ac}	6.35 ± 0.4^{Ad}	6.15 ± 0.2^{Ad}
Overall acceptability	Control	7.80 ± 0.3^{Aa}	7.54 ± 0.2^{Ab}	7.25 ± 0.3^{Ac}	6.95 ± 0.2^{Ad}	6.50 ± 0.3^{Be}	6.20 ± 0.4^{Bf}	5.90 ± 0.3^{Bg}
	Treatment	7.75 ± 0.2^{Aa}	7.62 ± 0.3^{Aab}	7.45 ± 0.2^{Ab}	7.15 ± 0.4^{Ac}	6.76 ± 0.3^{Ad}	6.44 ± 0.2^{Ae}	6.25 ± 0.4^{Ae}

Superscript a,b,c…f, shows the significant difference in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs. wrapped product. (N=6),



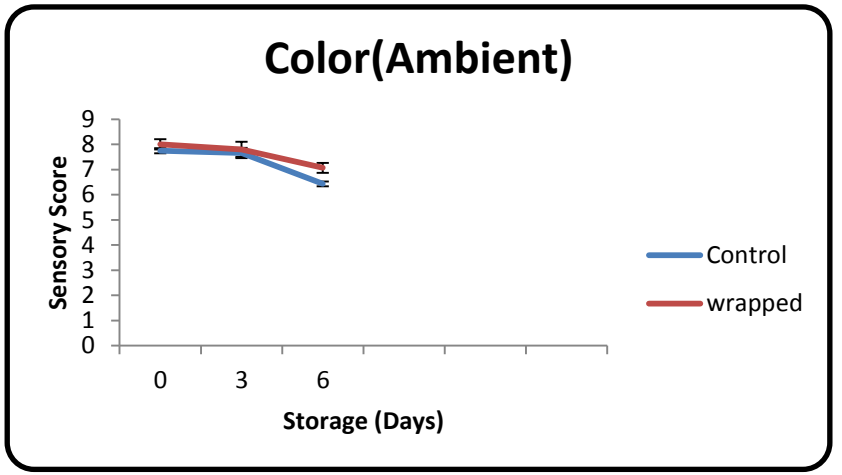
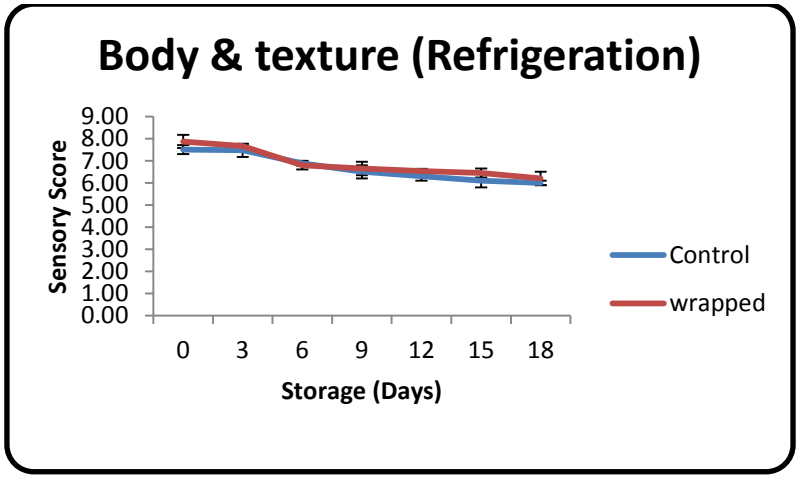


Fig. 4.3 Effect of storage on the sensory score of color at refrigeration & ambient temperature



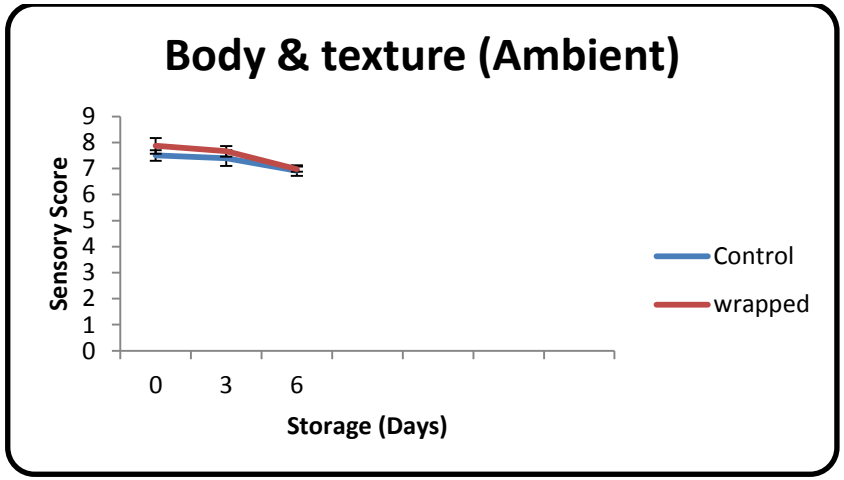
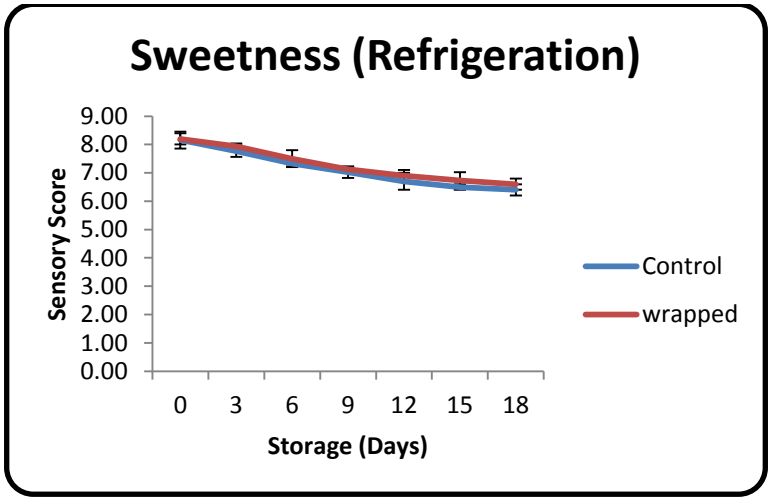


Fig. 4.4: Effect of storage on sensory scores of body and texture at refrigeration and ambient temperature



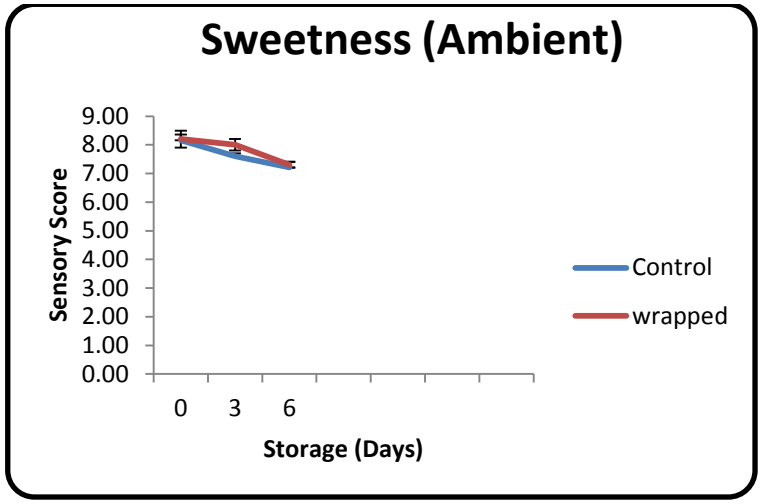
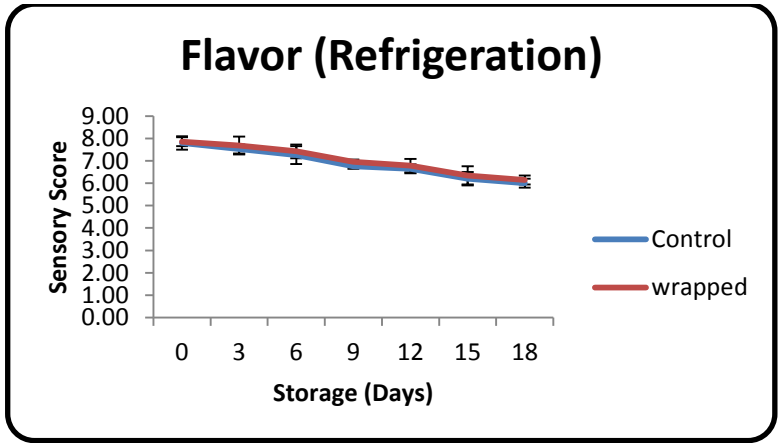


Fig 4.5: Effect of storage on the sensory score of sweetness at refrigeration & ambient temperature



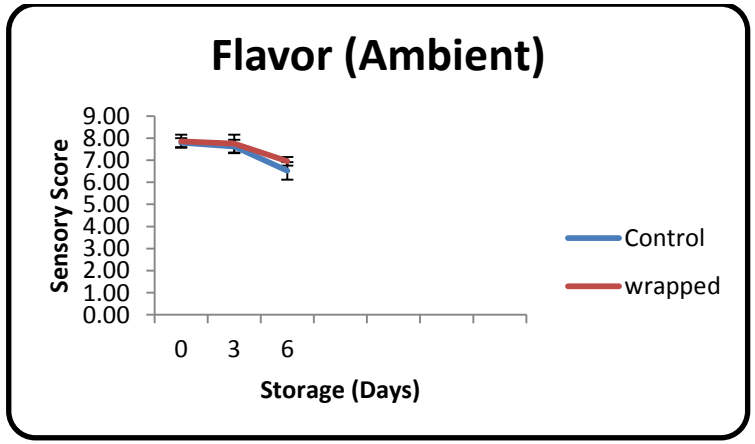


Figure 4.6: Effect of storage on the sensory scores of flavor at refrigeration & ambient temperature

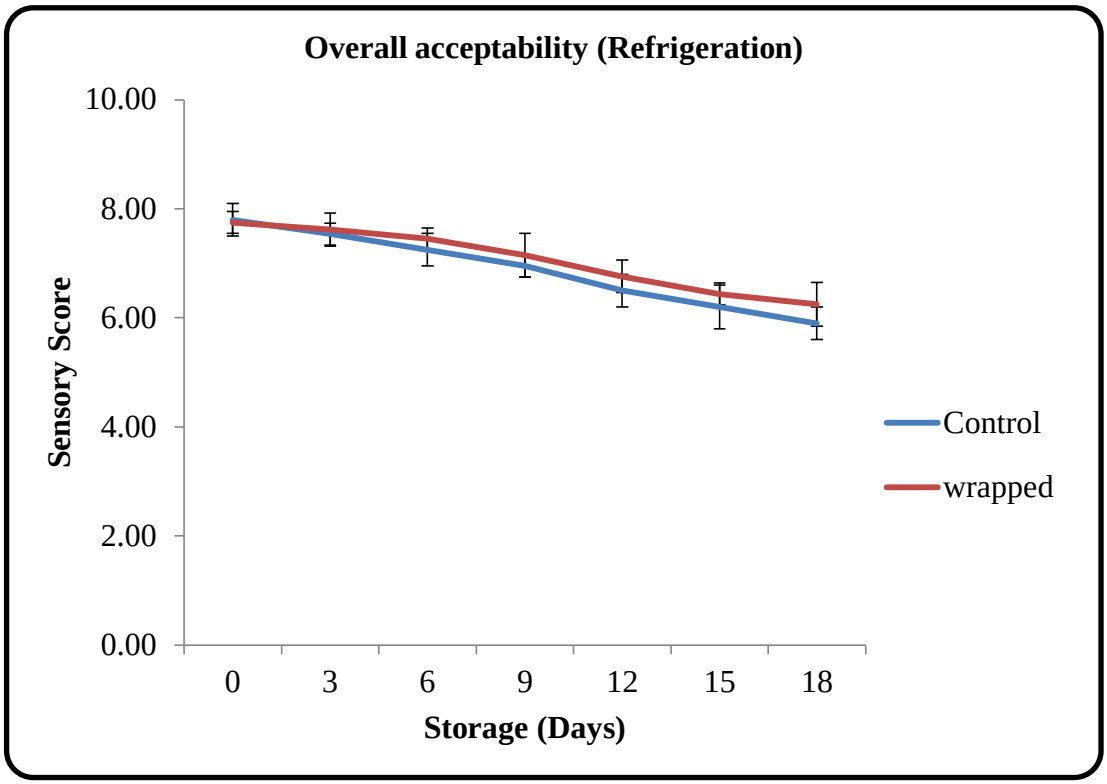
The body and texture score of control sample decreased from 7.50 to 6.92 while score of sample wrapped with edible film decreased from 7.87 to 6.98 after 6 days of storage at ambient temperature (Table 4.7 & Figure 4.4).Incase of storage at refrigeration temperature (4°C), the body & texture score of control sample decreased from 7.50 to 6.00 while score of sample wrapped in edible film decreased from 7.87 to 6.20 after 18 days of storage at refrigeration temperature (Table 4.8 &Figure 4.4). The decrease in score might be due to loss of moisture in the milk cake, it become hard which gave less acceptable body & texture as compared to fresh product or refrigerated product. At room temperature the integrity of grains remained intact, but the grains become harder and chewier in the product as the moisture content reduced. At refrigeration temperature (4°C), the product became dry, hard, sandy, and brittle which might be ascribed to the loss of moisture and possible crystallization of added sugar. The body & texture score decreased at both the temperatures & found significant as well between samples stored at ambient temperature during storage. Rao (2007) also reported the decrease in body and texture score of packed *Kalakand* during storage at 30ºC and 6 ºC. Similarly, Landge *et al* (2009) reported decrease in body and texture score during storage of milk cake packed in different packaging materials at 27ºC and 5ºC. Londhe *et al* (2012) also reported decrease in body and texture score during storage study of brown *Peda* at 30 ºC packed using different packaging materials.

Sweetness scores of the control sample decreased from 8.16 to 7.21 whereas, the score of milk cake with edible film also decreased from initial value of 8.20 to 7.30 at ambient temperature after 6 days of storage (Table 4.7 & Figure 4.5). On the other hand, the sweetness score of control sample decreased from 8.16 to 6.40 while, film wrapped with edible film decreased from 8.20 to 6.60 at refrigeration temperature (Table 4.8 & Figure 4.5). It can be inferred from the scores that though the decrease in sweetness scores of milk cake may be due to change in flavor profile (slightly bitter) of milk cake during the course of time. Also, the perceivable intensity of the product declined progressively, and could be attributed to personal sensory perception of an individual. Similarly, Chawla *et al* (2015) reported the decrease in sweetness score during the storage study of *dhoda burfi* at 30ºC.

Similarly, flavour scores of control sample decreased from 7.80 to 6.52 whereas, score of milk cake wrapped in edible film decreased from 7.86 to 6.95, after 6 days

storage at ambient temperature (Table 4.7 & Figure 4.6). On the other hand, control sample scores decreased from 7.80 to 6.00, while the wrapped sample showed significant decrease in flavor score from 7.86 to 6.15after 18 days storage at refrigeration temperature (Table 4.8 & Figure 4.6). The decrease in flavour scores may be attributed to slight losses of freshness, which is inherent with any food product during storage. This was also due to fat oxidation of the milk cake or due to the film after storage in the product, which gave slight bland flavour to the product being brittle afterwards. Sharma and Kulkarni (2003) also reported the decrease in mean flavour and body and texture scores of the control and MAP packaged *Malai Peda* samples in flexible packaging material at room temperature. Landge *et al* (2005) found that packaging materials significantly affect the flavour score of *Kalakand* during storage. Rao (2007) also reported the decrease in flavour score of *Kalakand* packed in different packaging materials during storage at 30ºC and 6 ºC. Landge *et al* (2009) reported decrease in flavour score during storage of packed Milk Cake at 27ºC and 5ºC.

Overall acceptability, average of various sensory parameters which further depends on several factors like proteolysis, lipolysis, and flavour changes score activity also decreased during storage. The control sample showed decrease in over acceptability score from 7.80 to 7.58 whereas, the overall acceptability score of milk cake wrapped in edible film decreased from 7.75 to 7.67 with 6 days of storage, at ambient temperature (Table 4.7 & Figure 4.7). On the other hand, control sample scores decreased from 7.80 to 5.90 and there was decreased in the scores of wrapped sample from 7.75 to 6.25 after 18 days storage at refrigeration temperature (Table 4.8& Figure 4.7). The product was sensory acceptable upto 6 days at ambient temperature (25°C) and up to 18 day (last day) of storage study at refrigeration temperature (4°C). Rao (2007) reported the decrease in overall acceptability score of *Kalakand* packed in different packaging materials during storage at 30ºC and 6ºC. Similar observations were recorded by Landge *et al* (2009) reported decrease in overall acceptability score during storage of packed milk cake at 27ºC and 5ºC. Londhe *et al* (2012) also reported decrease in overall acceptability score during storage study of brown *Peda* at 30ºC using different packaging materials. Chatli *et al* (2014) also observed significantly decrease in the scores for texture and overall acceptability with storage for chevon chunks wrapped in bioactive films impregnated with nisin and cinnamaldehyde.



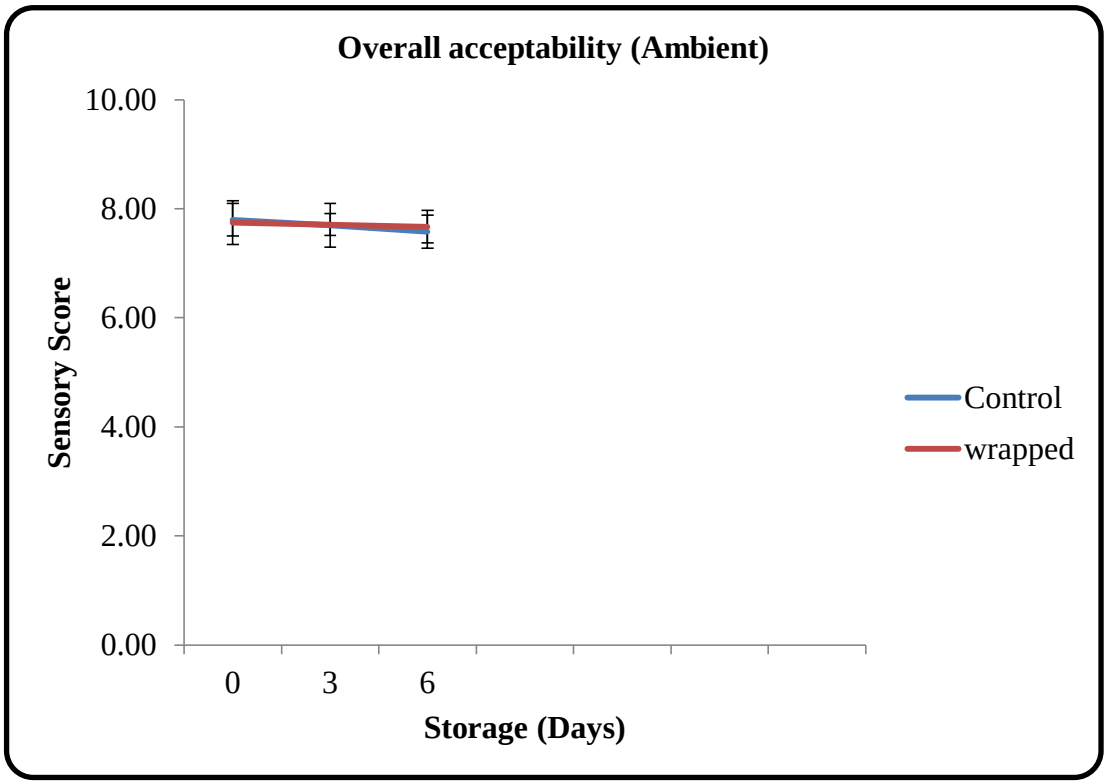


Figure 4.7: Effect of storage on the sensory scores of Overall acceptability at refrigeration (4°C) & ambient temperature (25°C)

4.3.2 Effect of storage on mechanical properties of film during storage

The properties of film wrapped on the milk cake were also analyzed for its physico-chemical and mechanical characteristics. For this, the film wrapped on the milk cake was firstly remove and then analyzed to visualize changes in various parameters

According to Gutierrez *et al* (2015[b]), a greater interaction between the starch and plasticizer could result in thicker films probably due to the formation of hydrogen bonds between the glycerol and starch. Figure 4.8 shows the gradual increase in the thickness of the film after the regular interval of time of 3 days at both refrigeration temperature (4°C) and ambient temperature (25°C). The thickness of film increased from 0.061 to 0.069 and 0.069mm (Table 4.9) at refrigeration (4°C) and ambient conditions (25°C), respectively and showed the significant difference in the thickness of the film. The increase in the thickness with storage period was due to the migration of the moisture of milk cake to film. With increase in the moisture, starch granules swell and resulted in increased the thickness of the film. Increase in the moisture content probably validates the interaction between the starch and plasticizer during gelatinization, which resulted in thicker films (Gutierrez *et al* 2015[b]).

Water solubility is one of the important factor for choosing a film for specific applications. Generally, the effect of solubility of film depends on the type of the compound and their concentration and their inherent hydrophillicity and hydrophobicity indices. It was expected that the hydrophilic compound should increase solubility and hydrophobic compounds would decrease water solubility. Storage at refrigeration (4°C) and ambient temperature (25°C) showed the decrease in the water solubility of the film as shown in figure 4.9. The water solubility of film decreased from 27.2 to 25.4% at both temperatures. There was a significant difference ($p \leq 0.05$) in the water solubility of film at both the temperatures. With the storage period, there was increase in the moisture content, which further enhanced the phase separation, which lead to more water binding in the film and ultimately decreased in solubility. Similar study was observed by (Bertuzzi *et al* 2007[b], Rindlav-Westling *et al* 1998) reported that starch films have extensive intermolecular association between starch chains, especially amylose chains. The extensive intermolecular association in starch films may contribute to the low solubility of the tapioca starch film.

Tearing strength gradually decreased with storage period at both the temperatures ambient (25°C) and refrigeration (4°C) as shown in Figure 4.10. The increase in degree

of crystallinity of the polymer matrix was directly related to high TS values (Van Soest *et al* 1996; Flores *et al* 2007). During the storage time, the tearing strength decreased from 8.29 to 8.17 and 8.06 N in regular interval of 3 days at both ambient (25°C) and refrigeration temperature (4°C), respectively (Figure 4.11). This was probably due to the increase in the moisture content. There was a significant difference in the tearing strength of film at both the temperature. With the storage at both temperatures, although film thickness increased with storage time, but there was breakdown of starch content in the film due to the moisture which led to the decrease in the tearing strength of the film. This might be due to the increase in the Relative humidity. According to Saberi *et al* 2017, with increase in the RH, TS decreased.

Puncturing strength of film decreased with storage at ambient (25°C) and refrigeration temperature (4°C). It decreased from 2.58 to 2.14 and 2.29 at ambient (25°C) and refrigeration temperature (4°C), respectively (Figure 4.10). Puncturing strength was strongly affected by the water activity of the film which further had direct relation with moisture content. With the storage, moisture in the film got increased which decreased the puncturing strength of the film (Gontard 1993)

The water vapor transmission rate (WVTR) is one of the most important properties studied in edible films, because one of the main functions of a food packaging is to prevent or reduce the transfer of moisture from the surrounding environment into the product. During storage, WVTR increased gradually with storage at ambient and refrigeration temperature (Figure 4.12). Its determination can predict the loss or gain of water by weight in food where films are applied. Moreover, the WVTR is affected by numerous factors, including: film thickness, water activity, humidity, concentration of components used in the formulation of the films. WVTR of the film increased with storage at ambient (25°C) and refrigeration temperature (4°C). There was significant difference ($p \leq 0.05$) in WVTR of film at both ambient (25°C) and refrigeration temperature (4°C). The WVTR decreased from 7.30 to 7.57 g/m^2h in regular interval of 3 days at both temperatures respectively. As the relative humidity of the environment increased, it led to the transfer of more water through the film which increases the water vapor transmission rate. An increase in humidity resulted in an increase in the WVTR. In terms of the barrier, an increase in the water molecule content will result in lower water vapour barrier properties (Cuq *et al* 1997).

Table 4.9: Effect of storage on properties of edible film at ambient (25°C) & refrigeration (4°C) temperature

Properties	Temp.	Days						
		0	3	6	9	12	15	18
Thickness (mm)	4°C	0.061±0.002^{a}	0.063±0.003^{a}	0.066±0.005^{a}	0.066±0.004^{b}	0.068±0.003^{b}	0.068±0.002^{c}	0.069±0.004^{d}
	25°C	0.061±0.003^{a}	0.067±0.004^{b}	0.069±0.002^{b}	-	-	-	-
Puncturing strength (N)	4°C	2.58±0.3^{a}	2.49±0.4^{b}	2.48±0.2^{bc}	2.47±0.3^{c}	2.38±0.4^{d}	2.31±0.2^{e}	2.29±0.3^{f}
	25°C	2.58±0.4^{a}	2.42±0.2^{b}	2.14±0.3^{c}	-	-	-	-
Tearing strength (N)	4°C	8.29±0.4^{a}	8.25±0.3^{ab}	8.22±0.5^{bc}	8.20±0.4^{cd}	8.16±0.3^{d}	8.09±0.3^{e}	8.06±0.4^{e}
	25°C	8.29±0.3^{a}	8.24±0.2^{b}	8.17±0.4^{c}	-	-	-	-
WVTR (g/m^2h)	4°C	7.3±0.2^{a}	7.32±0.3^{ab}	7.38±±0.2^{b}	7.43±0.3^{c}	7.47±0.2^{cd}	7.54±0.3^{e}	7.57±0.1^{e}
	25°C	7.3±0.2^{a}	7.45±0.3^{b}	7.57±0.2^{c}	-	-	-	-
Water solubility (%)	4°C	25.4±0.06^{e}	25.6±0.05^{e}	25.9±0.04^{de}	26.4±0.06^{c}	26.9±0.04^{b}	27±0.03^{ab}	27.2±0.05^{a}
	25°C	25.4±0.05^{a}	26.71±0.06^{a}	27.13±0.04^{a}	-	-	-	-
Water activity	4°C	0.514±0.006^{a}	0.54±0.005^{b}	0.54±0.006^{b}	0.55±0.004^{bc}	0.55±0.005^{bc}	0.56±0.004^{c}	0.56±0.005^{c}
	25°C	0.514±0.005^{a}	0.541±0.004^{b}	0.586±0.004^{c}	-	-	-	-
Moisture (%)	4°C	20.81±0.3^{e}	21.87±0.4^{e}	21.91±0.2^{cd}	21.97±0.4^{c}	22.02±0.3^{bc}	22.08±0.4^{b}	22.29±0.5^{a}
	25°C	20.81±0.4^{b}	21.12±0.3^{b}	22.29±0.2^{a}	-	-	-	-
Transmittance (%)	4°C	76±2.1^{a}	73.3±3.2^{b}	71.7±2.4^{bc}	70.3±3.1^{cd}	69.8±2.8^{d}	69.03±2.6^{d}	68.1±3.3^{d}
	25°C	76±2.5^{a}	75.72±2.9^{ab}	74.14±3.1^{b}	-	-	-	-

Superscript a,b,c…f, shows the significant difference ($p<0.05$) in data w.r.t storage days

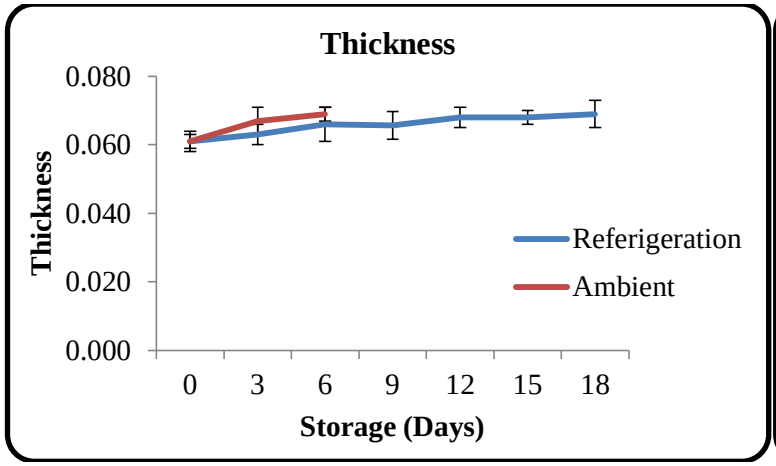


Figure 4.8: Effect of storage on film thickness

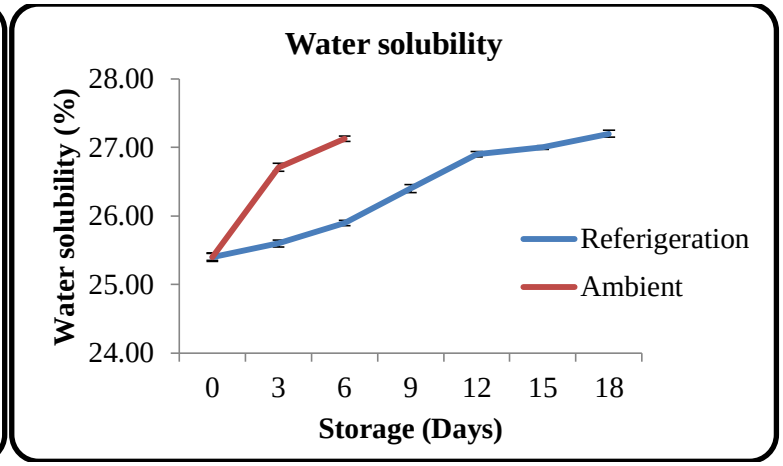


Figure 4.9: Effect of storage on the water solubility of film

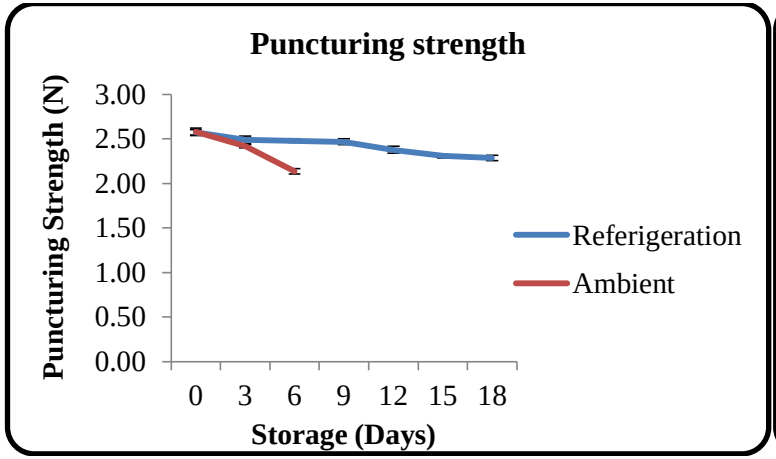


Figure 4.10: Effect of storage on Puncturing strength of film

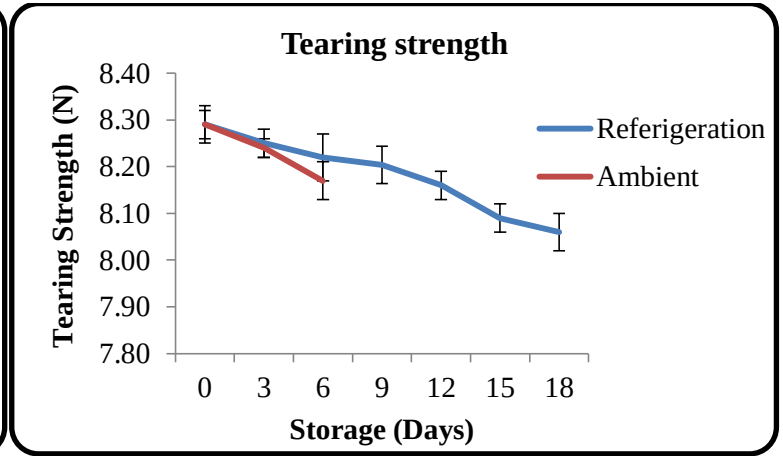


Figure 4.11. Effect of storage on Tearing strength of film

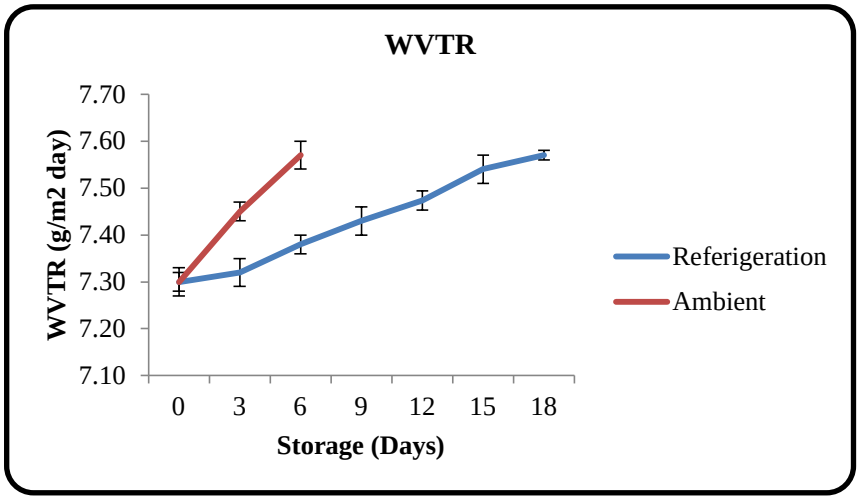


Figure 4.12: Effect of storage on WVTR of film

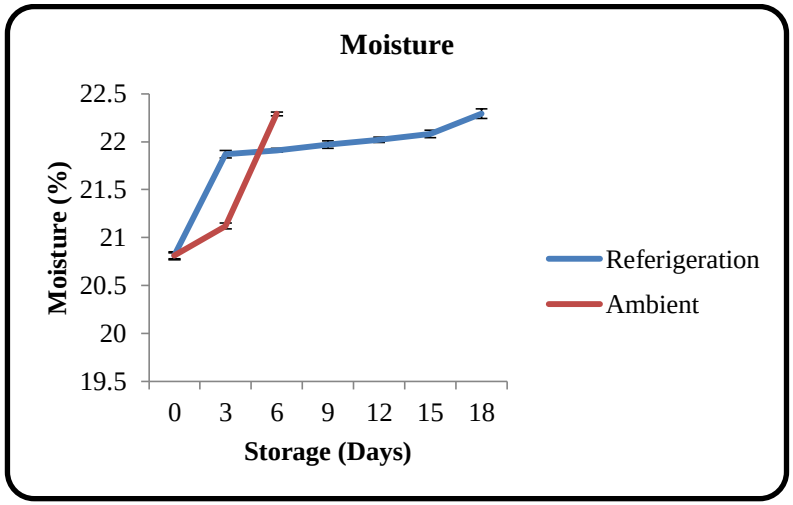


Figure 4.13: Effect of storage on moisture of film

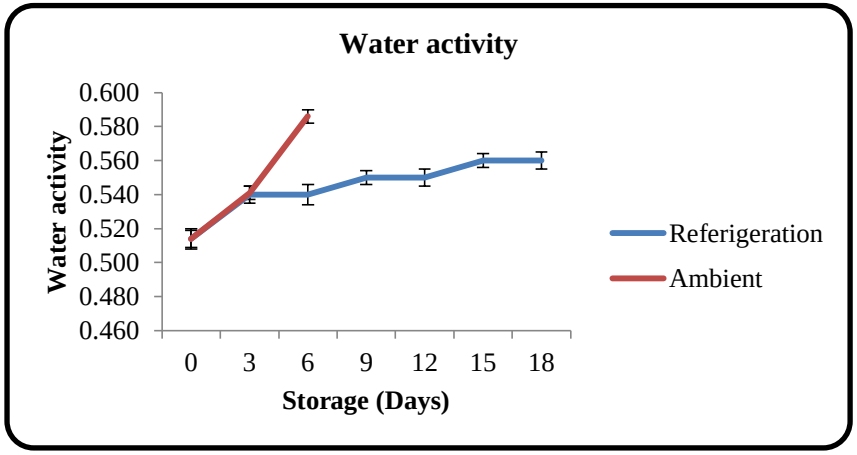


Figure 4.14: Effect of storage on Water activity of film

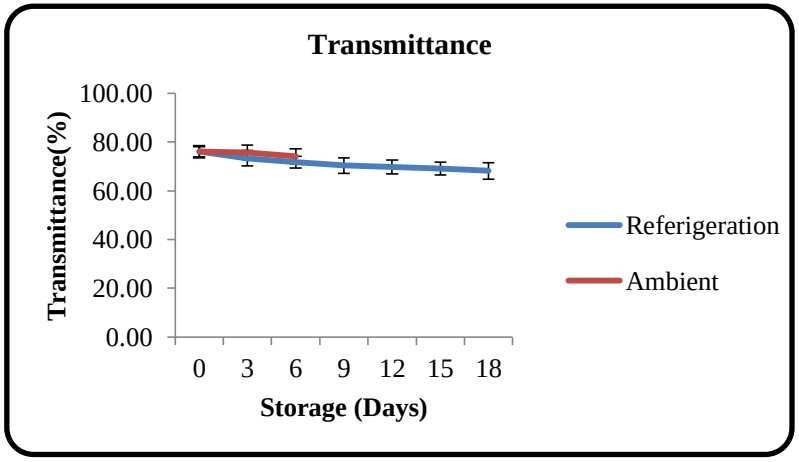


Figure 4.15: Effect of storage on Transmittance of film

Moisture of the film increased gradually with ambient and refrigeration storage (Figure 4.13). Moisture content of film increased from 20.81 to 22.29 and 22.29 per cent at ambient (25°C) and refrigeration temperature (4°C) respectively. There was a significant difference ($p \leq 0.05$) in moisture content of film at both ambient (25°C) and refrigeration temperature (4°C). According to Gutierrez *et al* (2015[a]), water activity is directly proportional to the moisture content of the edible films. Therefore, higher the values for the making flour-derived films were caused by a lower interaction between the glycerol and starch, which allowed water absorption from the environment. Also, with the storage of the film wrapped on milk cake, the moisture content was increased due to the transfer of moisture to the film. The fact is validated with increase in thickness of the film as well. It can be seen from the values that moisture content increased to 2.08%, with the progression of storage days of 18 days.

Water activity increased with storage at ambient (25°C) and refrigeration temperature (4°C). It directly depends on the moisture content of the film. It increased from 0.514 to 0.586 and 0.560 at ambient (25°C) and refrigeration temperatures (4°C) respectively (Figure 4.14). There was a significant difference ($p \leq 0.05$) in water activity of film at both the temperatures. The water activity has linear relation with the moisture content. As the moisture content of the film increased, the water activity of the film was also increased.

Transmittance decreased gradually with storage at ambient (25°C) and refrigeration temperature (4°C)**.** There was a significant difference ($p \leq 0.05$) in transmittance of film at both the temperatures. Transmittance decreased from 76.00 to 74.14 and 68.10 respectively (Figure: 4.15). Transmittance of the film gradually decreased with storage due to the increased of the thickness of the film.

4.3.3 Changes in physico-chemical parameters of milk cake wrapped with edible film during storage

Physico-chemical properties are the important parameters to know the stability of any food product during storage study. It gives an idea about the shelf life of the newly developed food products. Considering the above facts in present study, storage study of the milk cake wrapped with edible film was conducted to know the stability and shelf life of the product. For evaluating the changes during storage intervals in

physico-chemical properties of the product, samples were analyzed for changes in moisture, pH, ash, titratable acidity, HMF value, tyrosine value, free fatty acid, TBA value, Peroxide value and reducing sugars at the regular interval of 3 days at ambient temperature (25°C) and refrigeration temperature (4°C). There was a significant difference observed in all the physico-chemical parameters.

There was a decrease in moisture content of sample taken place at ambient temperature (25°C) as well as refrigeration temperature (4°C) with the progressed storage period (Figure 4.16). The decrease in moisture content during refrigerated storage might be due to drying at low temperature (4±2ºC) and surface evaporation (Sharma *et al* 2003). It showed the significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both the temperatures. The moisture of control sample decreased from 21.45 to 19.67 per cent whereas moisture of wrapped sample decreased from 23.97 to 21.93 per cent with the storage for 6 days at ambient temperature (Table 4.11 & Figure 4.16). On the other hand, the moisture of control sample decreased from 21.17 to 1.95 percent and it decreases from 23.97 to 23.22 percent for the wrapped sample with storage at refrigeration temperature (4°C) for 18 days (Table 4.10 & Figure 4.16). Sharma *et al* (2003) reported decrease in moisture during storage of *Peda* at 11±1ºC due to drying at low temperature. Venkatesh *et al* (2005) reported moisture loss during storage of *Kalakand* at 30±0.5ºC and 5±0.5ºC.

There was decrease in ash content of milk cake sample with storage at both the conditions. It showed the significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both the temperatures. The ash content of control sample decreased from 2.17 to 1.95 per cent whereas, ash content of treated sample decreased from 2.28 to 2.06 per cent with the storage at refrigeration temperature (4°C) for 18 days (Figure 4.17 & Table 4.11). Whereas in case of storage at ambient temperature (25°C) for 6 days, control sample decreased from 2.17 to 2.01 per cent and ash content decreased from 2.28 to 2.08 per cent for wrapped sample (Figure 4.17 & Table 4.11).

pH decreased as the storage period progressed at both of the temperatures. The decrease in pH of wrapped milk cake or unwrapped milk cake during storage may be due to microbial growth in dairy products which caused increase in acidity or decrease in pH. It shows the significant difference ($p \leq 0.05$) between the control

sample and wrapped sample at both the temperatures. pH of control sample decreased from 6.82 to 6.7 (Figure 4.18 & Table 4.10). Whereas it decreased from 6.78 to 6.63 in case of wrapped sample with storage at refrigeration temperature (4°C) for 18 days. Whereas it decreased from 6.82 to 6.52 for the control sample and in case of wrapped sample, it decreased from 6.78 to 6.5 with storage at ambient temperature (25°C) for 6 days (Figure 4.18 & Table 4.11). Kumar *et al* (1997) also reported a decrease in pH of *Peda* during storage at 20ºC. Londhe *et al* (2012) also reported decrease in pH of brown *Peda* during storage at 30ºC using different packaging techniques.

Increase in titratable acidity of sample took place at ambient temperature (25°C) as well as refrigeration temperature (4°C) as storage period progressed (Figure 4.19). The increase in acidity of sample during storage might be attributed to the production of lactic acid and other acids due to fermentation by microorganisms. There is a significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both temperatures. Titratable acidity increased from 0.39 to 0.54 per cent for control sample whereas it increased from 0.42 to 0.57 per cent LA for wrapped milk cake with edible with storage at ambient (25°C) for 6 days (Figure 4.19 & Table 4.11). On the other hand, titratable acidity increased from 0.39 to 0.51 per cent for control sample whereas, it increased from0.42 to 0.48 per cent for wrapped sample with storage at refrigeration temperatures (4°C) for 18 days (Figure 4.17 & Table 4.10). Increase in Acidity values were also reported by Sachdeva and Rajorhia (1982) in *Burfi*. Sharma *et al* (2003) reported increase in acidity of *Peda* during storage at 32±3ºC using different packaging materials. Karwasra *et al* (2003) also reported increase in acidity of Milk Cake during storage at 30±1ºC. Londhe *et al* (2012) also reported increase in acidity content of brown *Peda* during storage at 30°C using packaging techniques.

Increase in FFA of sample was observed at ambient temperature (25°C) as well as refrigeration temperature (4°C) as storage period progressed (Figure 4.20). This increase in FFA content is due to degradation of fat which is primarily affected by the growth of yeasts and molds. It was seen that there was statistical low significant difference ($P<0.05$) in FFA contents among storage periods indicating that the period of storage influenced the FFA formation to a great extent.

Table 4.10: Effect of storage on physico-chemical properties of milk cake with edible film at refrigeration temperature (4°C)

Properties	Sample	Days						
		0	3	6	9	12	15	18
Moisture (%)	Control	21.45 ± 1.2^{Aa}	21.35 ± 0.9^{Aa}	21.19 ± 1.3^{Aa}	21.09 ± 1.1^{Aa}	20.99 ± 1.2^{Aa}	20.95 ± 1.3^{Aa}	20.89 ± 0.8^{Aa}
	Treatment	23.97 ± 1.3^{Ba}	23.72 ± 0.8^{Ba}	23.63 ± 0.9^{Ba}	23.58 ± 1.1^{Ba}	23.36 ± 1.3^{Ba}	23.33 ± 0.9^{Ba}	23.22 ± 1.2^{Ba}
Ash (%)	Control	2.17 ± 0.2^{Aa}	2.11 ± 0.3^{Aa}	2.08 ± 0.1^{Aa}	2.06 ± 0.2^{Aa}	2 ± 0.1^{Aa}	1.98 ± 0.4^{Aa}	1.95 ± 0.3^{Aa}
	Treatment	2.28 ± 0.3^{Aa}	2.24 ± 0.1^{Aa}	2.20 ± 0.2^{Aa}	2.19 ± 0.4^{Aa}	2.15 ± 0.1^{Aa}	2.08 ± 0.3^{Aa}	2.06 ± 0.2^{Aa}
pH	Control	6.82 ± 0.1^{Aa}	6.78 ± 0.2^{Aab}	6.75 ± 0.1^{Aab}	6.73 ± 0.2^{Aab}	6.70 ± 0.2^{Abc}	6.70 ± 0.1^{Abc}	6.70 ± 0.2^{Ac}
	Treatment	6.78 ± 0.2^{Aa}	6.75 ± 0.1^{Aa}	6.70 ± 0.2^{Aa}	6.69 ± 0.1^{Aa}	6.67 ± 0.2^{Aa}	6.66 ± 0.2^{Aa}	6.63 ± 0.2^{Aa}
Titratable Acidity (%)	Control	0.39 ± 0.02^{Aa}	0.42 ± 0.03^{Aab}	0.45 ± 0.02^{Aab}	0.45 ± 0.04^{Abc}	0.48 ± 0.06^{Abc}	0.51 ± 0.04^{Abc}	0.54 ± 0.02^{Ac}
	Treatment	0.42 ± 0.03^{Aa}	0.45 ± 0.04^{Aa}	0.48 ± 0.03^{Aa}	0.48 ± 0.02^{Aa}	0.51 ± 0.04^{Aa}	0.51 ± 0.05^{Aa}	0.57 ± 0.03^{Aa}
FFA (µeq/g)	Control	0.24 ± 0.020^{Aa}	0.24 ± 0.030^{Aa}	0.29 ± 0.04^{Ab}	0.32 ± 0.03^{Abc}	0.34 ± 0.02^{Acd}	0.37 ± 0.031^{Ad}	0.42 ± 0.04^{Ae}
	Treatment	0.24 ± 0.02^{Aa}	0.24 ± 0.04^{Aa}	0.29 ± 0.030^{Ab}	0.32 ± 0.030^{Ac}	0.34 ± 0.04^{Ac}	0.38 ± 0.02^{Ad}	0.40 ± 0.02^{Ad}
HMF (µ moles/100g)	Control	18.97 ± 0.2^{Aa}	19.11 ± 0.3^{Aa}	19.89 ± 0.4^{Aa}	21.37 ± 0.1^{Ab}	23.74 ± 0.3^{Ac}	24.14 ± 0.2^{Ac}	25.78 ± 0.2^{Ad}
	Treatment	18.38 ± 0.3^{Aa}	18.67 ± 0.4^{Aa}	19.12 ± 0.1^{Aa}	20.79 ± 0.3^{Ab}	22.13 ± 0.2^{Ab}	23.85 ± 0.1^{Ac}	25.12 ± 0.2^{Ac}
TBA (OD at 532 nm)	Control	0.237 ± 0.02^{Aa}	0.243 ± 0.03^{Aa}	0.247 ± 0.01^{Aa}	0.249 ± 0.02^{Aa}	0.255 ± 0.03^{Aa}	0.264 ± 0.01^{Aa}	0.269 ± 0.02^{Aa}
	Treatment	0.231 ± 0.01^{Aa}	0.24 ± 0.03^{Aa}	0.242 ± 0.02^{Aa}	0.238 ± 0.02^{Aa}	0.253 ± 0.01^{Aa}	0.261 ± 0.03^{Aa}	0.265 ± 0.02^{Aa}
Tyrosine Value (mg/100g)	Control	0.22 ± 0.005^{Aa}	0.22 ± 0.01^{Aa}	0.25 ± 0.007^{Ab}	0.27 ± 0.008^{Ab}	0.32 ± 0.005^{Ac}	0.3 ± 0.01^{Ac}	0.32 ± 0.005^{Ac}
	Treatment	0.22 ± 0.01^{Aa}	0.23 ± 0.005^{Aab}	0.25 ± 0.006^{Abc}	0.27 ± 0.005^{Ac}	0.31 ± 0.007^{Ad}	0.32 ± 0.006^{Bd}	0.33 ± 0.01^{Ad}
Peroxide Value (meq of O_2/kg fat)	Control	0.13 ± 0.01^{Aa}	0.15 ± 0.005^{Aa}	0.17 ± 0.006^{Ab}	0.22 ± 0.005^{Ac}	0.25 ± 0.007^{Ad}	0.26 ± 0.006^{Ade}	0.27 ± 0.005^{Ae}
	Treatment	0.14 ± 0.005^{Aa}	0.15 ± 0.01^{Aa}	0.17 ± 0.007^{Ab}	0.21 ± 0.008^{Ab}	0.24 ± 0.005^{Ac}	0.25 ± 0.01^{Ad}	0.27 ± 0.005^{Ae}
Reducing sugars (%)	Control	18.85 ± 0.2^{Aa}	19.1 ± 0.3^{Aab}	19.19 ± 0.1^{Aab}	19.3 ± 0.4^{Aab}	19.4 ± 0.3^{Aab}	19.47 ± 0.2^{Aab}	19.64 ± 0.1^{Ab}
	Treatment	18.95 ± 0.3^{Aa}	19.10 ± 0.2^{Aab}	19.25 ± 0.3^{Aab}	19.28 ± 0.1^{Aab}	19.28 ± 0.4^{Aab}	19.37 ± 0.2^{Aab}	19.47 ± 0.3^{Ab}

Superscript a,b,c…e, shows the significant difference in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs. wrapped product. (N=3)

Table 4.11: Effect of storage on physico-chemical properties of milk cake with edible film at ambient temperature (25°C)

Properties	Sample	Days		
		0	3	6
Moisture (%)	Control	21.45 ± 1.2^{Aa}	20.21 ± 0.9^{Aab}	19.67 ± 0.8^{Ab}
	Treatment	23.97 ± 0.9^{Ba}	22.34 ± 1.3^{Bab}	21.93 ± 1.1^{Bb}
Ash (%)	Control	2.17 ± 0.2^{Aa}	2.06 ± 0.3^{Aa}	2.01 ± 0.1^{Aa}
	Treatment	2.28 ± 0.1^{Aa}	2.19 ± 0.1^{Aa}	2.08 ± 0.1^{Aa}
pH	Control	6.82 ± 0.2^{Aa}	6.67 ± 0.1^{Aab}	6.52 ± 0.2^{Ab}
	Treatment	6.78 ± 0.1^{Aa}	6.64 ± 0.2^{Aab}	6.50 ± 0.1^{Ab}
Titratable Acidity (%)	Control	0.39 ± 0.03^{Aa}	0.48 ± 0.02^{Aa}	0.51 ± 0.03^{Aa}
	Treatment	0.42 ± 0.02^{Aa}	0.45 ± 0.04^{Aa}	0.48 ± 0.02^{Aa}
FFA (µeq/g)	Control	0.24 ± 0.02^{Aa}	0.28 ± 0.04^{Ab}	0.39 ± 0.03^{Ac}
	Treatment	0.24 ± 0.02^{Aa}	0.27 ± 0.03^{Ab}	0.36 ± 0.02^{Bc}
HMF (µ moles/100g)	Control	18.98 ± 0.3^{Aa}	21.67 ± 0.4^{Aa}	22.88 ± 0.2^{Aa}
	Treatment	18.38 ± 0.2^{Ba}	20.28 ± 0.3^{Bab}	22.12 ± 0.2^{Ab}
TBA (OD at 532 nm)	Control	0.237 ± 0.02^{Aa}	0.248 ± 0.03^{Aab}	0.261 ± 0.02^{Ab}
	Treatment	0.231 ± 0.03^{Aa}	0.241 ± 0.01^{Aa}	0.254 ± 0.02^{Aa}
Tyrosine value (mg/100g)	Control	0.22 ± 0.007^{Aa}	0.25 ± 0.005^{Aa}	0.32 ± 0.01^{Aa}
	Treatment	0.22 ± 0.01^{Aa}	0.24 ± 0.005^{Aa}	0.29 ± 0.008^{Aa}
Peroxide Value (meq of O_2/kg fat)	Control	0.13 ± 0.01^{Aa}	$0.19\pm.005^{Aa}$	0.33 ± 0.008^{Ab}
	Treatment	0.14 ± 0.007^{Ba}	0.18 ± 0.005^{Aa}	0.29 ± 0.01^{Ab}
Reducing sugars (%)	Control	18.85 ± 0.2^{Aa}	20.12 ± 0.3^{Ab}	21.83 ± 0.1^{Ac}
	Treatment	18.95 ± 0.3^{Ba}	20.07 ± 0.2^{Bb}	21.72 ± 0.2^{Ac}

Superscript a,b,c shows the significant difference in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs. wrapped product. (N=3),

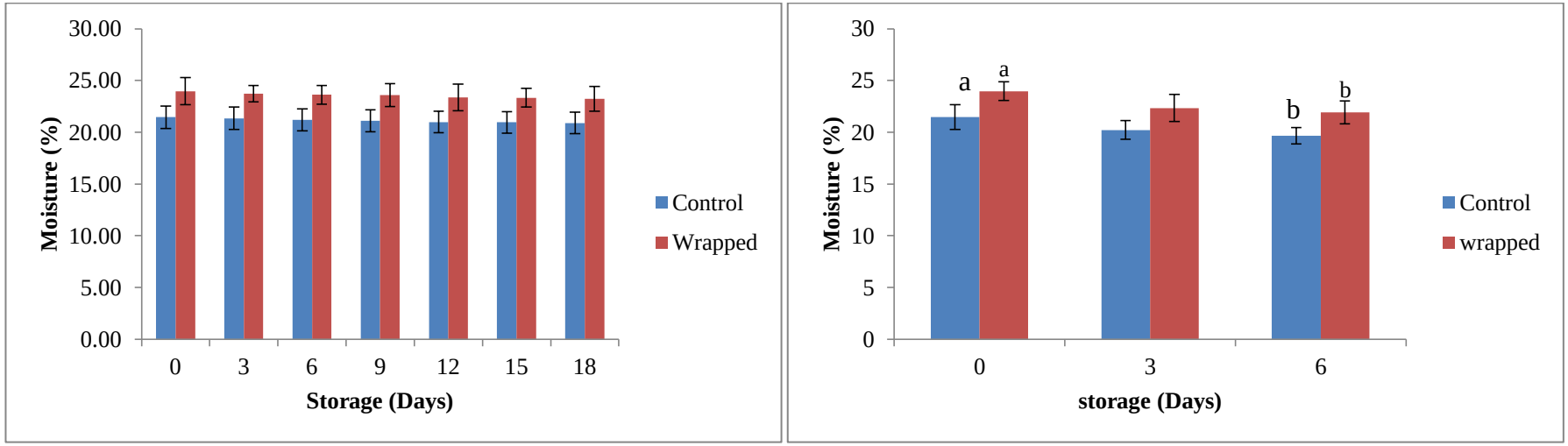


Figure 4.16: Effect of storage on moisture at refrigeration (4°C) and ambient temperature (25°C)

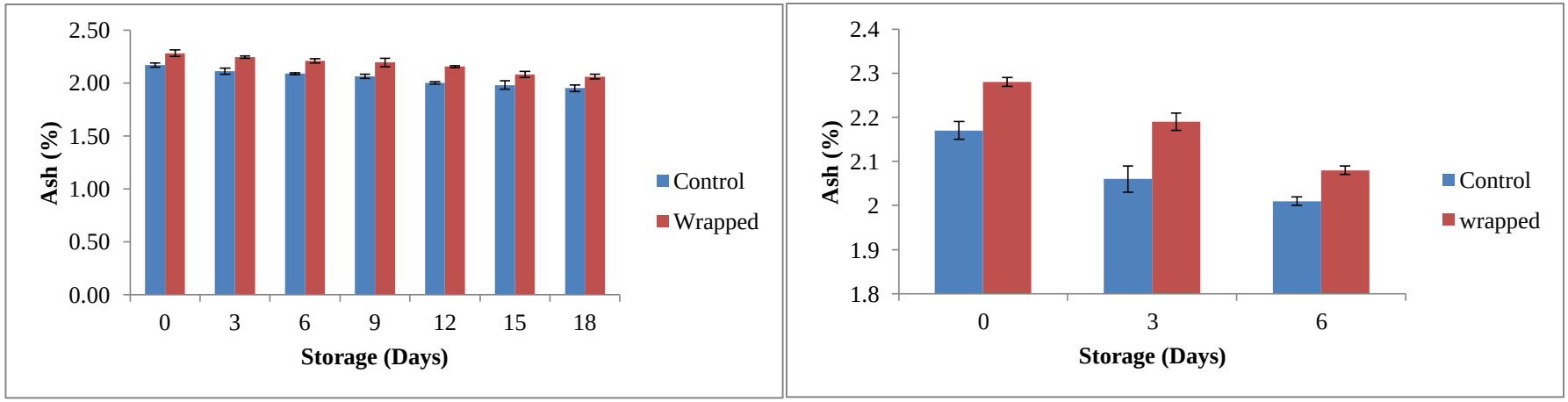


Figure 4.17: Effect of storage on ash at refrigeration (4°C) and ambient temperature (25°C)

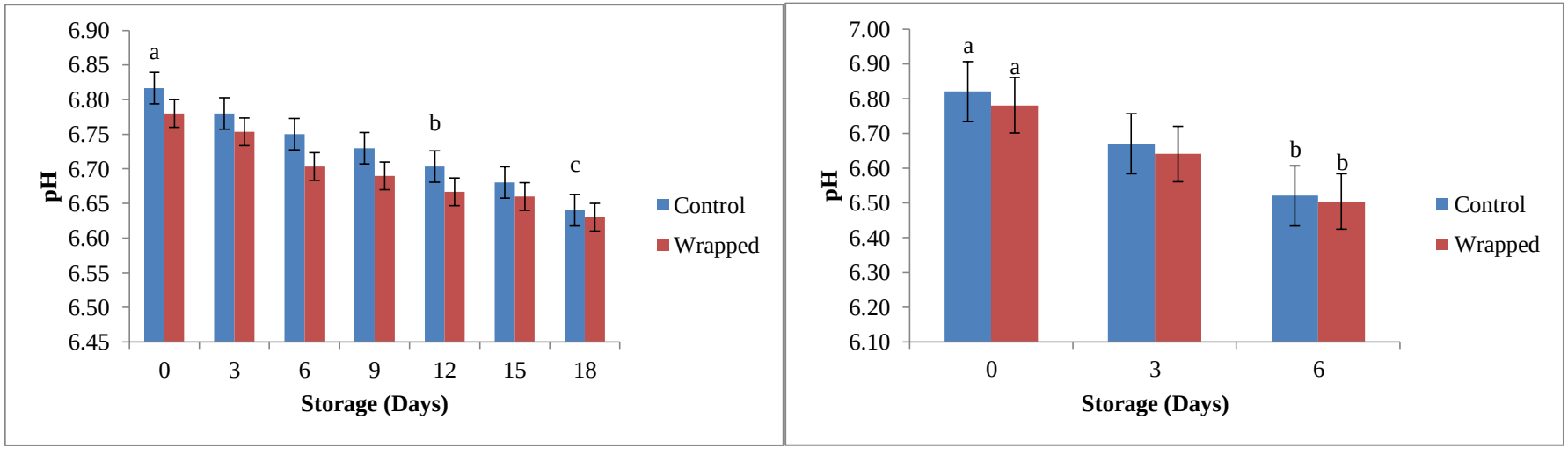


Figure 4.18: Effect of storage on pH at refrigeration (4°C) and ambient temperature (25°C)

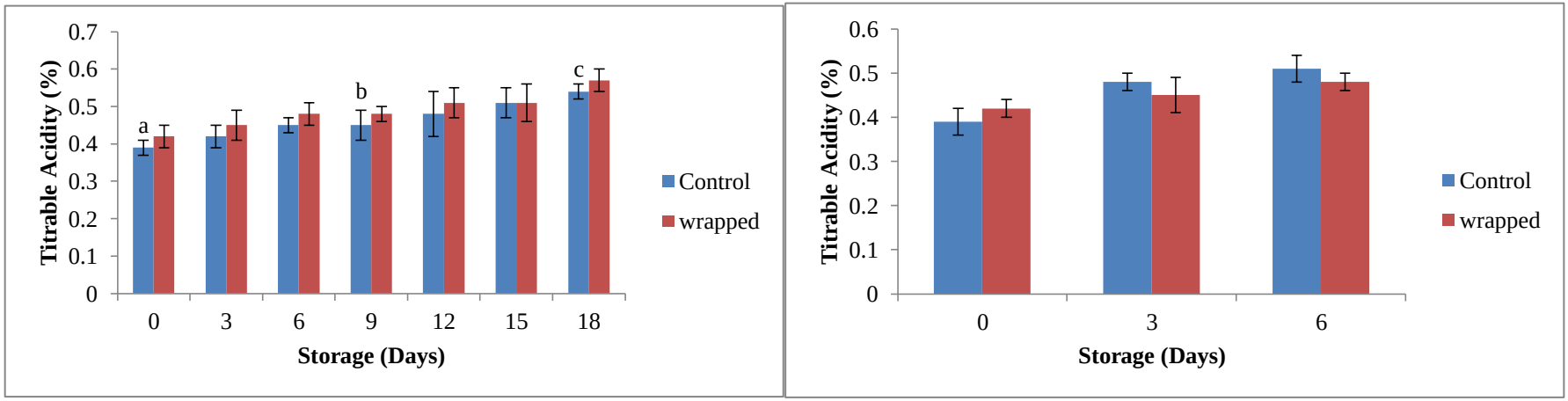


Figure 4.19: Effect of storage on Titratable acidity at refrigeration (4°C) and ambient temperature (25°C)

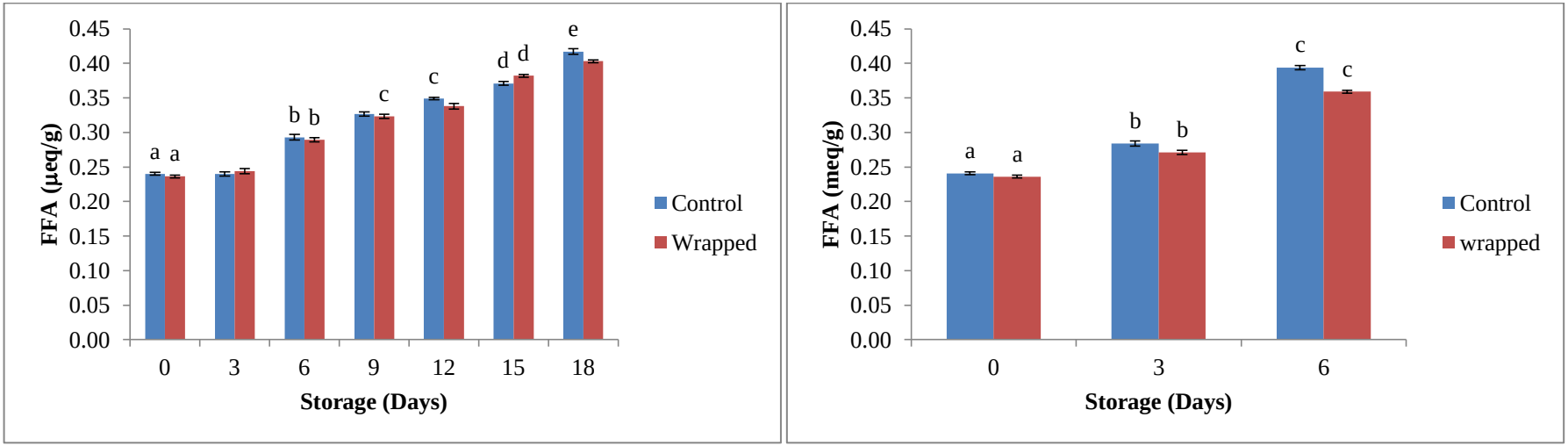


Figure 4.20: Effect of storage on FFA at refrigeration (4°C) and ambient temperature (25°C)

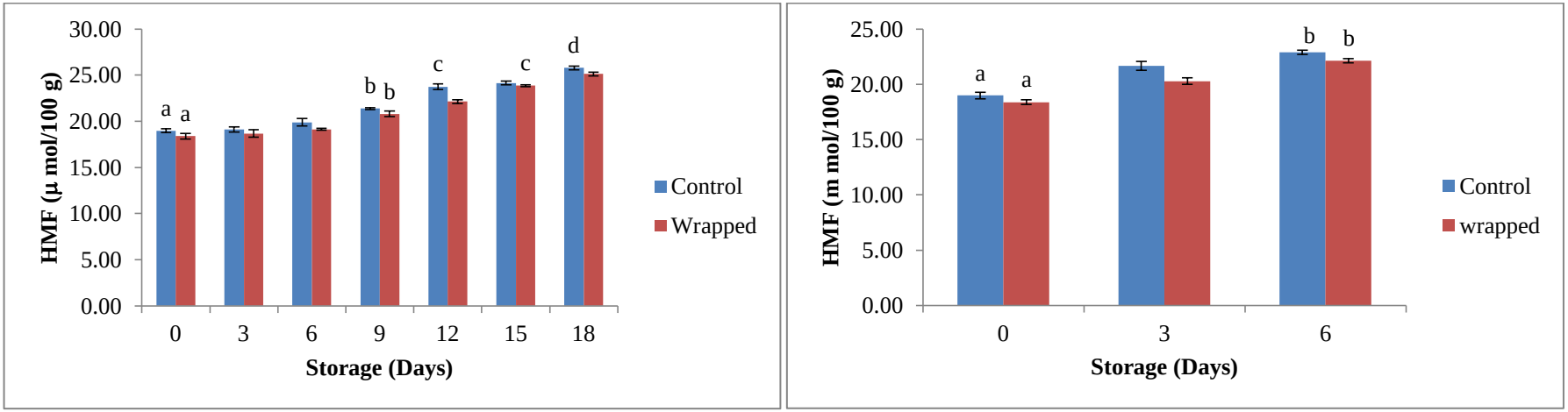


Figure 4.21: Effect of storage on HMF at refrigeration (4°C) and ambient temperature (25°C)

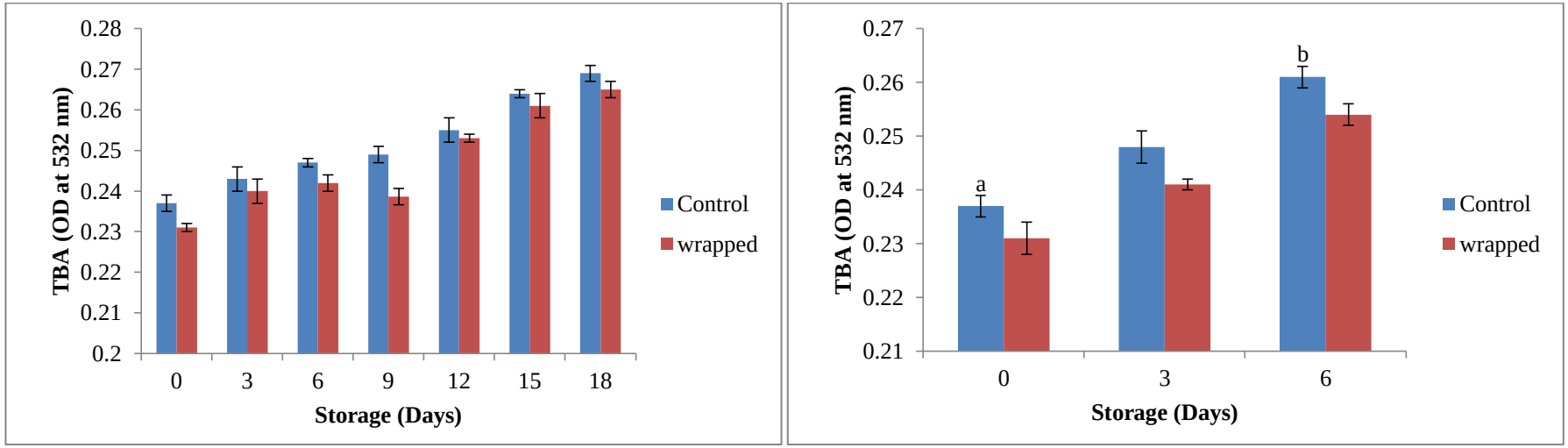


Figure 4.22: Effect of storage on TBA at refrigeration (4°C) and ambient temperature (25°C)

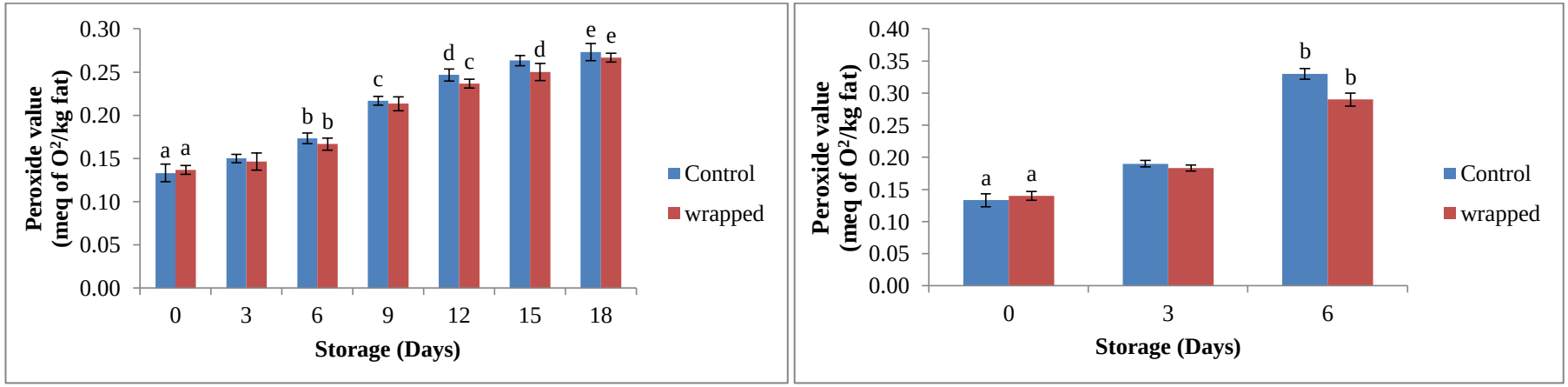


Figure 4.23: Effect of storage on peroxide value at refrigeration (4°C) and ambient temperature (25°C)

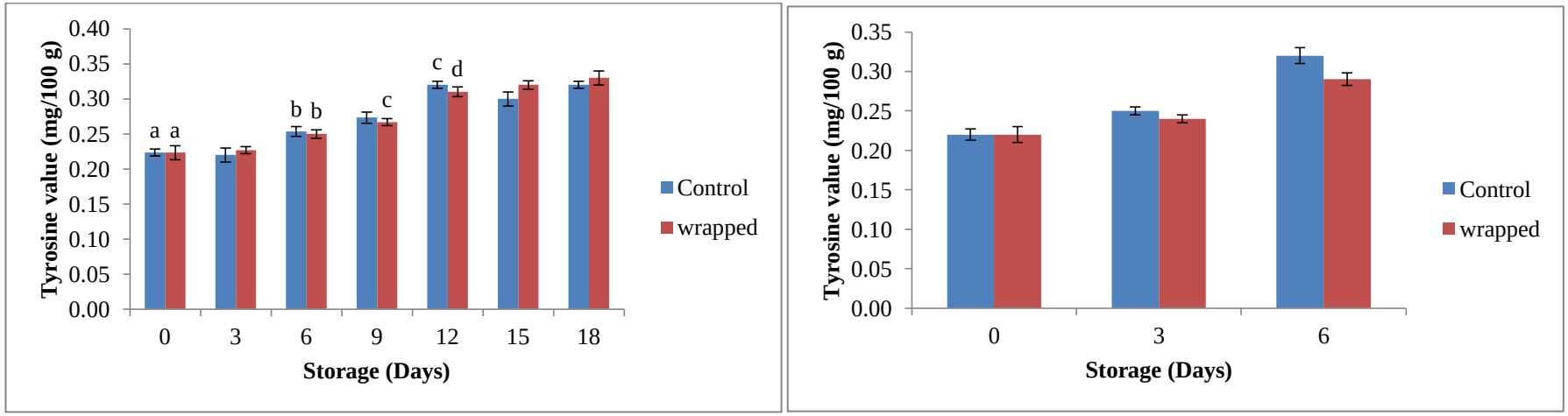


Figure 4.24: Effect of storage on Tyrosine value at refrigeration (4°C) and ambient temperature (25°C)

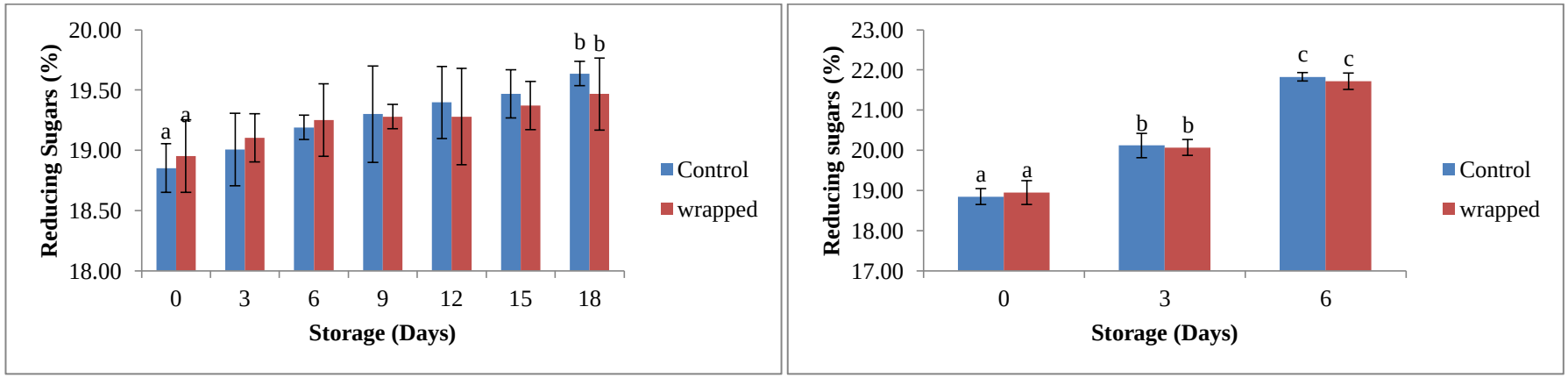


Figure 4.25: Effect of storage on reducing sugars at refrigeration (4°C) and ambient temperature (25°C)

FFA of control sample increased from 0.24 to 0.42 µeq/g whereas it increased from 0.24 to 0.40 µeq/g for wrapped milk cake with edible film with storage at refrigeration temperature (4°C) for 18 days (Figure 4.20 & Table 4.10). On the other hand, it increased from 0.24 to 0.39 µeq / g for control sample whereas FFA of wrapped film increased from0.24 to 0.36 µeq / stored at ambient temperature (25°C) for 6 days (Figure 4.20 & Table 4.11). Biradar *et al* (1985) recorded in the studies of *Peda* that the FFA content increased in LDPE package with advances in storage period. Vijaykhader and Patel (1983) also reported changes in free fatty acids in *Peda* during storage at ambient temperature (25ºC),

Increase in HMF content of milk cake was observed at ambient (25°C) temperature as well as refrigeration temperature (4°C) as the storage period progressed (Figure 4.21). Such increase in HMF content in heat treated milk products is an usual phenomenon but could be restricted by controlling factors such as storage temperature, storage period and certain specific characteristics of the product viz. pH, TS and a_w etc. (Walstra and Jenness 1984). It showed the significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both the temperatures. The HMF value increased from 18.97 to 25.78 µ moles/100g for control sample whereas it increased from 18.38 to 25.12 µ moles/100g for wrapped sample stored at ambient temperature (25°C) after 6 days (Figure 4.21 & Table 4.11). whereas HMF value increased from 18.97 to 22.8 for control sample while it increased from 18.38 to 22.12 µ moles/100g for wrapped sample at refrigeration temperature (4°C) after 18 days (Figure 4.21 & Table 4.10). Increase in HMF content during storage were also recorded by Patil and Pal (2005) and Sachdeva and Rajorhia (1982) in *Burfi* & by Kumar *et al* (1997) and Sharma *et al* (2003) in *Peda*.

TBA increased with storage at both ambient (25°C) and refrigeration temperature (4°C). It could be seen from the Table (Table 4.10 & 4.11) that the TBA values of milk cake wrapped in edible film during storage period increased at ambient temperature (25°C) than at refrigeration temperature (4°C). It shows the significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both refrigeration (4°C) and ambient temperature (25°C). The TBA value increased from 0.237 to 0.261 OD at 532 nm for the control sample whereas it increased from 0.231 to 0.254 OD at 532 nm for wrapped sample stored at ambient temperature (25°C) for 6 days (Figure 4.22 & Table 4.11). Whereas TBA increased from 0.237 to 0.269 OD

at 532 nm for control sample and it increased from 0.231 to 0.265 OD at 532 nm for wrapped sample stored at refrigeration temperature (4°C) for 18 days (Figure 4.22 & Table 4.10). The increase in TBA values might be due to oxidative and hydrolytic rancidity of milk fat of milk cake during storage. This suggests that most probably the varying quantities of atmospheric oxygen might have reacted with the unsaturated fatty acids of the lipids of milk cake, resulting in different TBA values. Increases in TBA values were also reported by Sachdeva and Rajorhia (1982) in *Burfi* and Karwasra *et al* (2003) in milk Cake during storage period.

Peroxide value is a measure for the autoxidation of fat. Higher peroxide value signifies the oxidative deterioration of fat. PV measures the amount of hydro peroxides formed as primary oxidation products at the initial stages of lipid oxidative reactions (Teets and Were 2008) Unsaturated fatty acids in milk fat constitute the major target for oxidation. Oxidation of fat may lead to a multitude saturated and unsaturated compounds resulting in oxidative rancidity. With storage at ambient (25°C) and refrigeration temperature (4°C), the peroxide value of control sample increased from 0.13 to 0.33 meq of O_2/kg fat whereas it increased significantly from 0.14 to 0.29 meq of O_2/kg fat for wrapped sample stored at ambient temperature (25°C) for 6 days (Figure 4.23 & Table 4.11). Whereas it increased from 0.13 to 0.27 meq of O_2/kg fat for control sample and increased from 0.14 to 0.27 meq of O_2/kg fat for wrapped sample at refrigeration temperature (4°C) for 18 days (Figure 4.23 & Table 4.10).

The tyrosine value was used as to measure the extent of proteolysis in stored Milk cake samples. Proteolysis is the enzymatic hydrolysis of peptide linkages of protein to produce small protein fragments, i.e. proteose – peptone, peptides and amino acids. With storage at ambient (25°C) and refrigeration temperature (4°C), the tyrosine value of control sample increased significantly from 0.22 to 0.29 mg/100g for control sample and increased from 0.22 to 0.32 mg/100g for wrapped sample at ambient temperature (25°C)for 6 days (Figure 4.24 & Table 4.11). On the other hand, tyrosine value increased from increased from 0.22 to 0.32 mg/100g for control sample and it increase from 0.22 to 0.33 mg/100g for wrapped sample at refrigeration temperature (4°C) for 18 days (Figure 4.24 & Table 4.10). Kumar (1999) reported tyrosine value of 40.30 and 31.45 mg/100 g product in market and laboratory made

samples respectively. These values are higher as compared to values in the present study.

Sugars can be found naturally in foods, including fruits and dairy products, in addition to those sugars that are added to foods during processing and added sugars are sugars that are not naturally found in the food product and are added during the production of the food (Erickson and Salvin 2015). There was increase in reducing sugars of sample took place at room temperature as well as refrigeration temperature (4°C) as storage period progressed. It shows the significant difference ($p \leq 0.05$) between the control sample and wrapped sample at both refrigeration (4°C) and ambient temperature (25°C). The reducing sugar value of increased from 18.85 to 21.83 per cent for control sample whereas it increased from 18.95 to 21.72 per cent for wrapped sample at ambient temperature (25°C) for 6 days (Figure 4.25 & Table 4.11). On the other hand, reducing sugars of wrapped sample increased from 18.85 to 19.64 per cent for control sample whereas it increased from 18.95 to 19.47 per cent and it at refrigeration temperature (4°C) for 18 days (Figure 4.25 & Table 4.10).

4.3.4 Effect of storage on microbiology properties of milk cake wrapped by edible film

Milk cake wrapped with edible film was analyzed for SPC, Yeast & mold and coliform. It was observed that the SPC and yeast & mold increased gradually with storage at both ambient (25°C) and refrigeration temperature (4°C). The growth at ambient temperature (25°C) is more prominent to refrigeration temperature (4°C). There was coliform growth at the last day of ambient (25°C) & refrigeration temperature (4°C).

According to the refrigeration (4°C) and ambient (25°C) storage, the SPC increased due to the growth of bacteria with the passage of time (Figure 4.26 and Table 4.12 & 4.13). There is a significant difference in control and the sample wrapped in edible film at ambient (25°C) and refrigeration conditions (4°C). The SPC of control sample increased from 3.56 to 5.41 log cfu/g and in case of wrapped sample, it increased from 3.63 to 5.30 log cfu/g with storage at ambient temperature (25°C) (Figure 4.26 and Table 4.13). On the other hand, SPC for sample control with edible film increased from 3.56 to 5.44 log cfu/g and from 3.63 to 5.24 log cfu/g for wrapped sample at refrigeration temperature (4°C) (Figure 4.26 and Table 4.12).

Table 4.12: Effect of storage on microbiological properties (log_{10} cfu/g) of milk cake with edible film at refrigeration temperature (4°C)

Properties	Sample	Days						
		0	3	6	9	12	15	18
SPC (log_{10} cfu/g)	Control	3.56 ± 0.03^{Be}	3.80 ± 0.05^{Ade}	4.03 ± 0.04^{Ad}	4.35 ± 0.02^{Ac}	4.63 ± 0.03^{Abc}	4.92 ± 0.05^{Ab}	5.44 ± 0.04^{Aa}
	wrapped	3.63 ± 0.02^{Ae}	3.73 ± 0.03^{Bde}	3.98 ± 0.04^{Ad}	4.27 ± 0.03^{Bc}	4.5 ± 0.02^{Bbc}	4.81 ± 0.04^{Bb}	5.24 ± 0.02^{Ba}
Y & M (log_{10} cfu/g)	Control	0.00	0.00	2.15 ± 0.05^{Ac}	2.23 ± 0.07^{Abc}	2.41 ± 0.06^{Ab}	2.76 ± 0.05^{Aa}	2.81 ± 0.05^{Aa}
	wrapped	0	0	2.07 ± 0.06^{Ab}	2.11 ± 0.05^{Bb}	2.19 ± 0.07^{Bb}	2.57 ± 0.05^{Bab}	2.83 ± 0.06^{Aa}
Coliform (log_{10} cfu/g)	Control	0.00	0.00	0.00	0.00	0.00	0.00	1.71 ± 0.05^{Aa}
	wrapped	0.00	0.00	0.00	0.00	0.00	0.00	1.51 ± 0.04^{Aa}

Superscript a,b,c…e, shows the significant difference in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs. wrapped product. (N=3)

Table 4.13: Effect of storage on microbiological properties (log_{10} cfu/g) of milk cake with edible film at ambient temperature (25°C)

Properties	Sample	Days		
		0	3	6
SPC (log_{10} cfu/g)	Control	3.56 ± 0.3^{Ac}	4.73 ± 0.2^{Ab}	5.41 ± 0.4^{Aa}
	Wrapped	3.63 ± 0.2^{Bc}	4.66 ± 0.3^{Bb}	5.33 ± 0.2^{Ba}
Y & M (log_{10} cfu/g)	Control	0.00	2.20 ± 0.05^{Aa}	2.76 ± 0.07^{Aa}
	Wrapped	0.00	2.17 ± 0.06^{Aa}	2.52 ± 0.05^{Ba}
Coliform (log_{10} cfu/g)	Control	0.00	0.00	1.55 ± 0.06^{Aa}
	Wrapped	0.00	0.00	1.45 ± 0.05^{Aa}

Superscript a,b,c shows the significant difference in data w.r.t storage days whereas A, B, C shows significant difference in data w.r.t control vs wrapped product. (N=3)

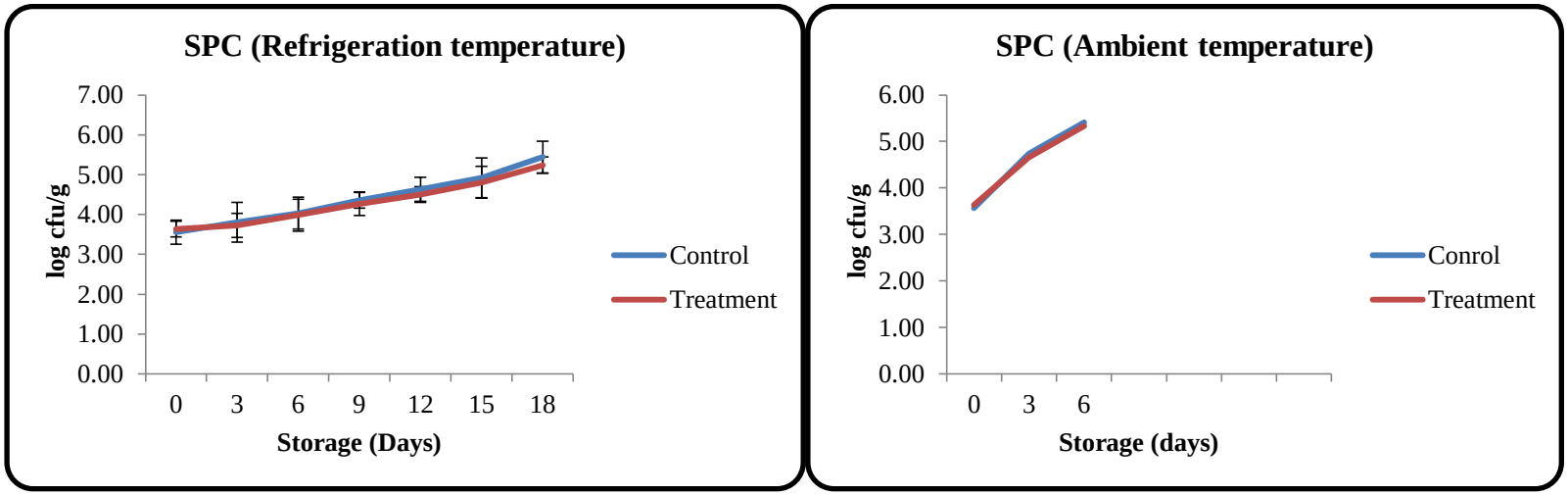


Figure 4.26: Effect of the storage on the SPC at refrigeration (4°C) and ambient temperature (25°C)

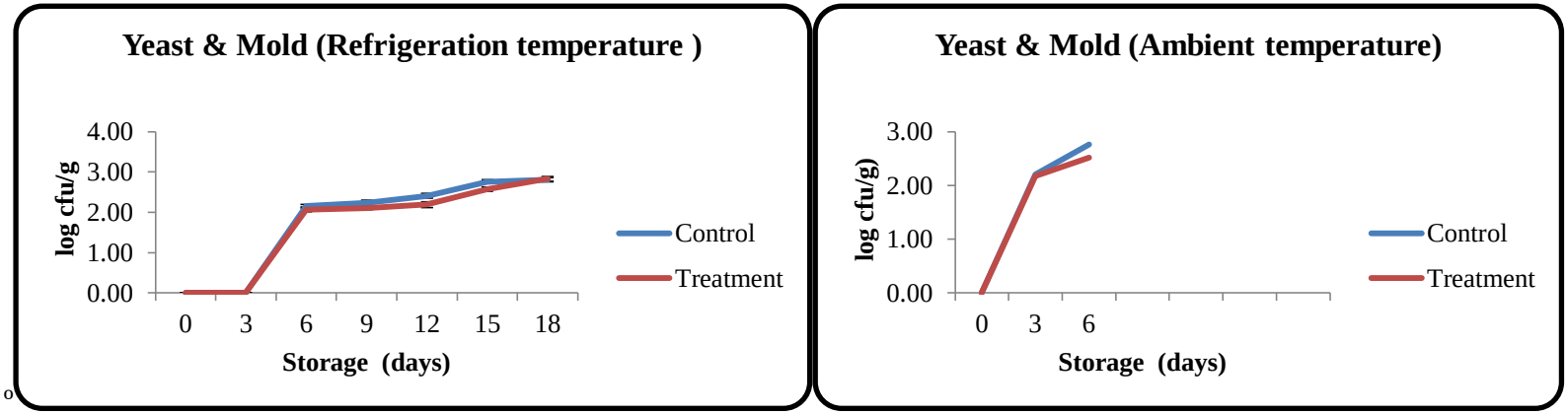


Figure 4.27: Effect of the storage on the Yeast & Mold at refrigeration (4°C) and ambient temperature (25°C)

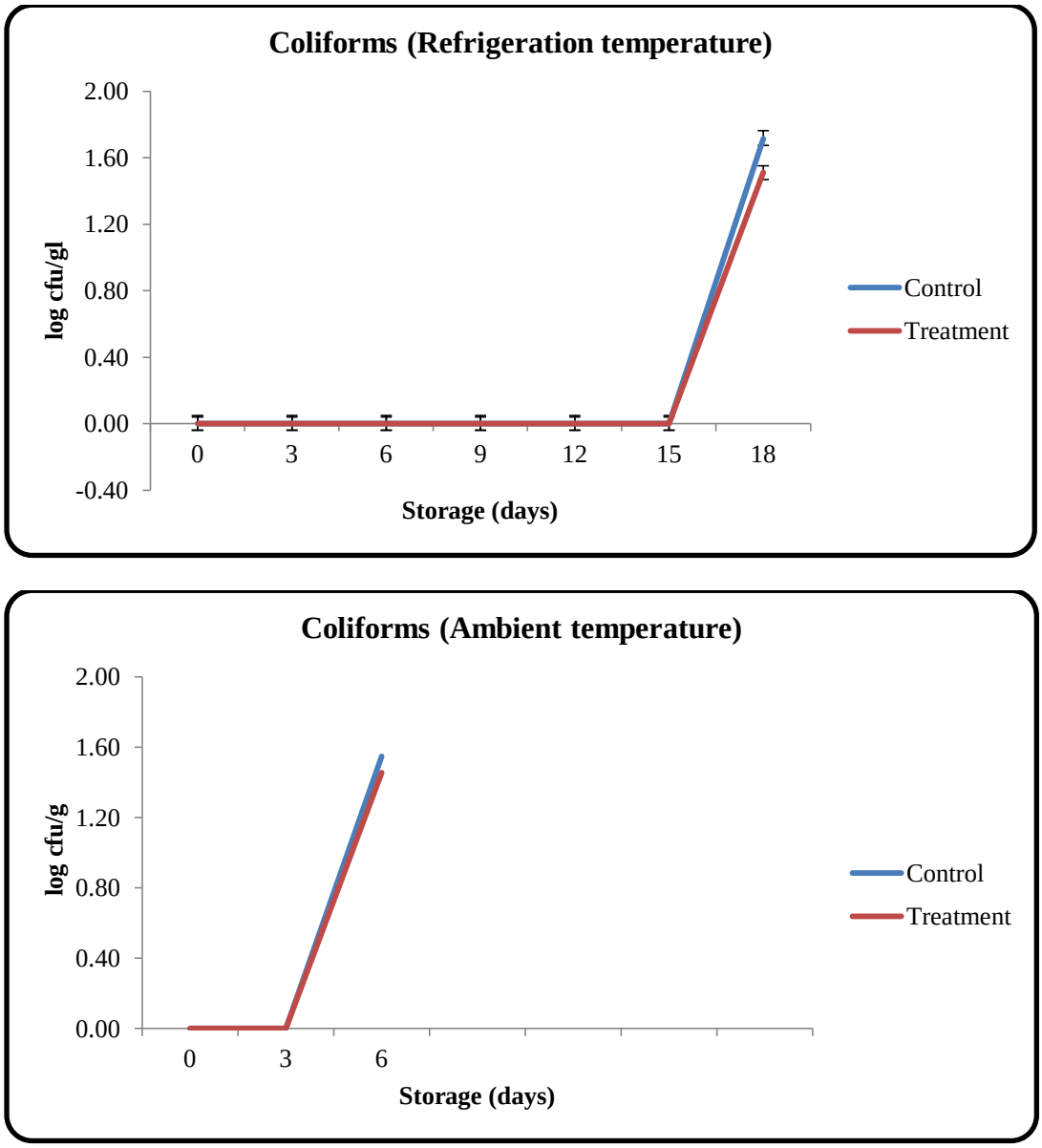


Figure 4.28: Effect of the storage on the Coliforms at refrigeration (4°C) and ambient temperature (25°C)

Basch *et al* (2011) have previously reported the antimicrobial activity of edible films based on tapioca starch and HPMC and containing nisin. They informed that this film produced a rapid decrease of the inoculated *L. innocua*, reaching a population 5 log cycles lower than the films without antimicrobials at the end of the storage. Sachdeva and Rajorhia (1982) reported increase in SPC during storage of Burfi at 30ºC and 50ºC. Other workers also reported increasing standard plate counts of Burfi during storage (Garg *et al* 1987). Londhe *et al* (2012) also reported increase in SPC of brown *Peda* during storage at 30ºC using different packaging techniques.

Yeast & mold count increased from 0 to 2.81 log cfu/ml for control sample and increased from 0 to 2.83 log cfu/g for wrapped sample in refrigeration storage (4°C) at 18th day (Figure 4.27 and Table 4.12). and SPC increased from 0 to 2.76 log cfu/g for control sample and increased from 0 to 2.52 log cfu/g for wrapped sample at 6th day in ambient temperature (25°C) (Figure 4.27 and Table 4.13). There is a significant difference in control and the sample wrapped in edible film at ambient (25°C) and refrigeration conditions (4°C). Moreira *et al* (2011) also reported a significant antimicrobial action of edible chitosan and casein polymers on yeast and mould counts of cheese and salam. Landge *et al* (2012) who observed minimum increase in yeast and mold count in Milk Cake when packed in aluminium foil with LDPE laminates during storage at 5ºC and 27ºC. Venkatesh *et al* (2005) also found lower yeast and mold count in *Kalakand* packed in aluminium foil. Londhe *et al* (2012) also reported increase in yeast and mold count of brown *Peda* during storage at 30°C using different packaging techniques.

There was no growth of coliform till 3rd day of ambient temperature (25°C) and 15th day at refrigeration temperature (4°C). Growth started at 6th day i.e. 1.55 log cfu/g for control sample & 1.45 log cfu/g for wrapped sample at ambient temperature (25°) (Figure 4.28 and Table 4.13). and 18th day of refrigeration temperature (4°C), coliform growth was 1.71 log cfu/g for control sample & 1.51 log cfu/g for wrapped sample (Figure 4.28 and Table 4.12). The coliform bacteria present in raw milk were destroyed during boiling during milk cake preparation as observed zero coliform count in fresh product. The presence of coliforms indicates the post production contamination of the finished product. Landge *et al* (2012) also reported increase in coliform count during storage of Milk Cake at 27°C and 50°C using different packaging materials. Londhe *et al* (2012) also reported increase in coliform count of brown *Peda* during storage at 70°C using different packaging techniques

4.4 Consumer acceptability studies of optimized product.

Consumer acceptability of any newly developed food product is one of the most important parameters to be taken under consideration for exploring the potential marketability of the product. Consumer acceptance studies thus play a key role in

decision making for the launch of a newly developed product in the market. In present study to evaluate the potential of the developed product for marketability a pilot consumer study was conducted. For consumer study optimized product was prepared as per the procedure described in Figure 3.1. A total number of 110 prospective consumers, representing either sex and of varying age groups, education level and income group from the faculty members, campus of GADVASU and PAU and common people of proximate areas were selected. The consumer's response/ comments were recorded on a predefined Performa supplied along with the sample. The results are presented in Table 4.14.

Table 4.14 Frequency distribution of consumer acceptance (preference) of the milk cake with edible film

Milk cake with edible film		Degree of Liking			
		Like extremely	Like very much	Like moderately	Like slightly
No. of consumer	110	43	55	8	4
Percent of total respondents	100	39.09	50	7.27	3.64

It is evident from the Fig.4.29 that out of total people who participated in the consumer study, 39.09 per cent of them rated the product 'Liked extremely' while 50 per cent rated it as 'Liked very much' and 7.27 per cent rated as 'Liked moderately'. Out of 110 Consumer, only 3.64 per cent consumers liked slightly the product and provided the fact that they don't like eating milk cake with edible film. So, it was the mindset of the consumers which didn't allow them to provide good sensory scores. However, consumers from all age groups liked the product very much (Fig. 4.29) which means that a satisfactory performance for the product was obtained and therefore, it is safe to conclude that the developed product has potential for wider marketability.

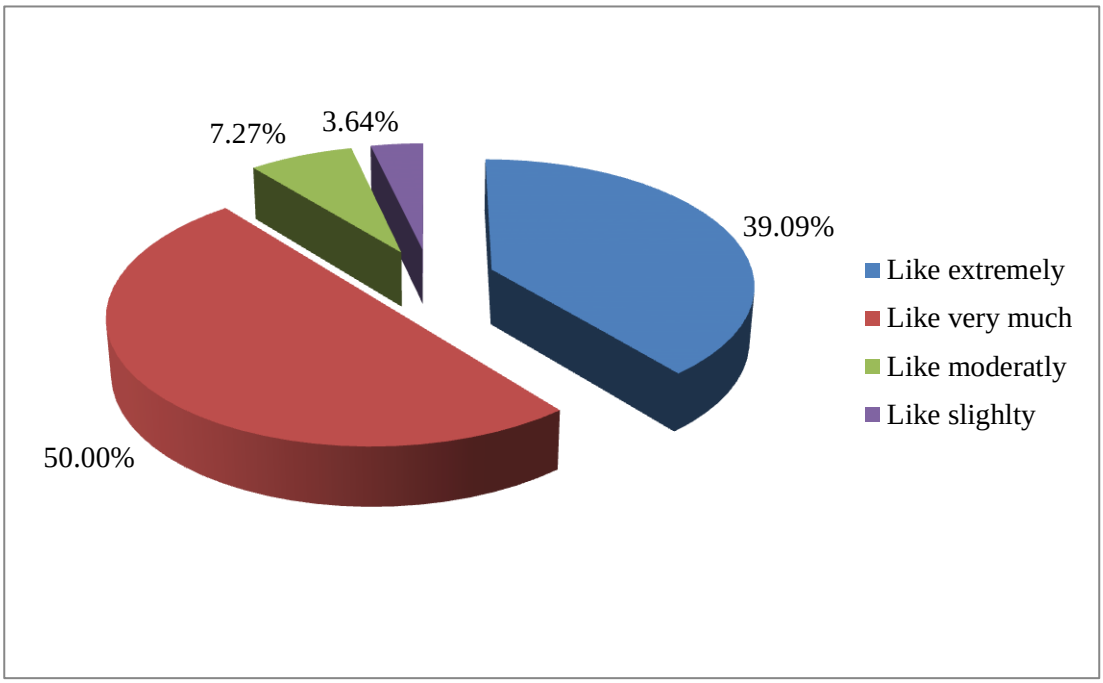


Figure 4.29: Consumer acceptability study of optimized product

4.4.1Consumer acceptability on the basis of age group

People from different age groups were selected for the consumer study. These age groups are categorized into 4 groups i.e. people less than 20, between 20-30, between 30-40 and more than 40 years. These consumers of different age group showed their interest towards the product as shown in Figure 4.30.

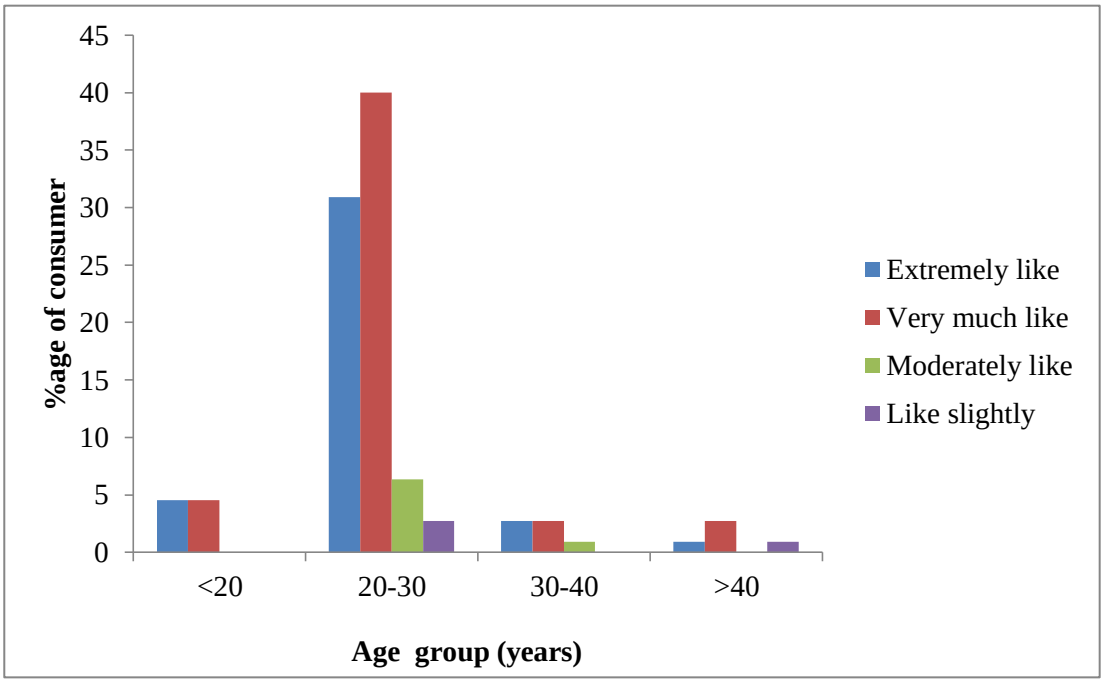


Figure 4.30: Consumer acceptability on the basis of age group

4.4.2 Consumer acceptability on the basis of income group

The consumer acceptability was also analyzed by the people of different income group. There are three income group i.e. people with income less than 1.5 lacs, between 1.5 to 5 lacs and people more than 5 lacs income. Their interest towards the product is shown in Figure 4.31.

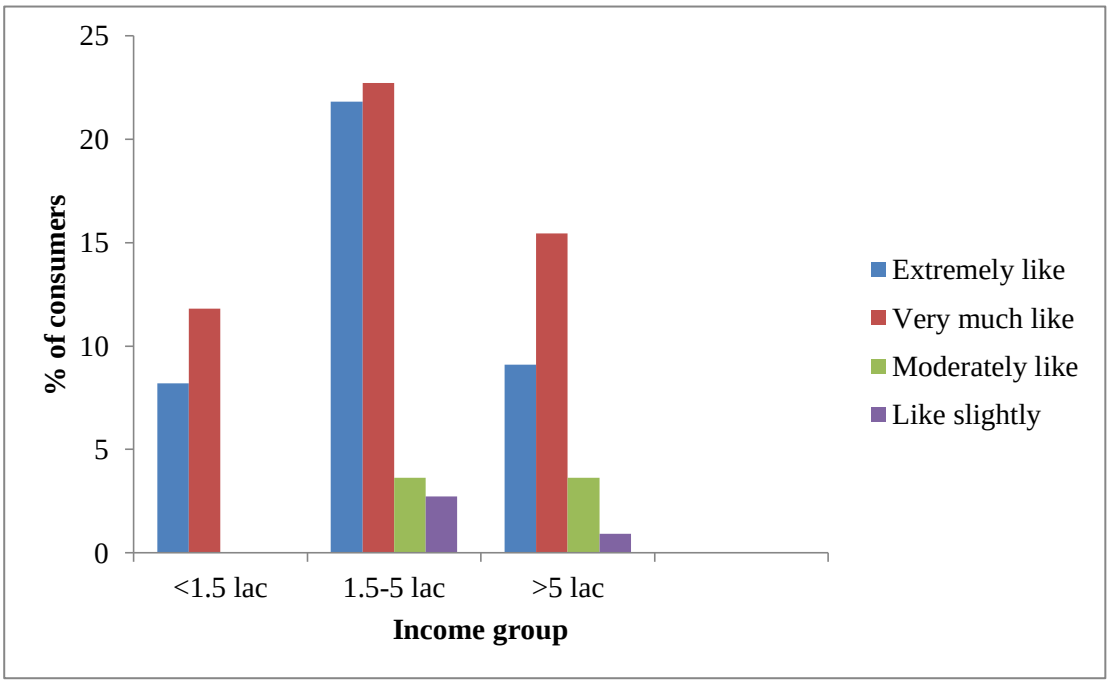


Figure 4.31: Consumer acceptability on the basis of income group

CHAPTER – V

SUMMARY AND CONCLUSIONS

The project was undertaken considering extensive load on environment for packaging of one of the most desirable items of today`s era i.e. sweetmeats produced from milk or milk-based products. Thus, present investigation was carried out for the development and characterization of edible film for packaging of milk cake with a systematic approach towards the packaging of the food products. The details of the given project are as follow:

5.1 Screening of the polymers and plasticizers

For preparation of edible films, from a huge variety of available base materials and endless list of plasticizers, some easily available and abundantly used options like corn, wheat & rice starch and protein sources such as soy protein isolate & sesame protein isolate were tried along with glycerol, mannitol & sorbitol as a plasticizer. Various preliminary trials were conducted and yielded results in the form of flexibility of film and ease with which film can be made out of these options. The trials were conducted with varying range of carbohydrate based polysaccharide from 4 to 6% (starches such as corn, wheat & rice) and protein such as sesame protein isolate & soy protein isolate. On the other hand, to increase the flexibility of the film, different plasticizers such as glycerol, mannitol & sorbitol at varying range from 2 & 3 % were checked for their suitability. Different temperature time conditions were also tried for the drying off the film & finally 40°C for 16-17 hours was decided, to get desirable characteristics of film. Time temperature combination plays an important role in formation of film. High temperature for short time gave the film formation with somewhat wrinkled structure which was not acceptable & finally as 40°C for 16-17 hours was decided, to get desirable characteristics of film. Results indicated that edible film prepared from corn, wheat and rice starch and glycerol as a plasticizer, at a concentration of 5% and 2% respectively, can yield a film with desired characteristics.

5.2 Optimization of the starch and glycerol

To further screen the best starch amongst options mentioned above, and to optimize the level of the same films from corn, wheat & rice starch were prepared @ 5% and 2% of glycerol. The starch was optimized by analyzing the physico-chemical

& mechanical and microbiological properties of the edible film. Different properties such as thickness, tearing strength, puncturing strength, WVTR, water solubility, moisture, water activity, transmittance, color were analyzed to find out the best results of film. The structural integrity was also considered to check out the best structure of film prepared from these three starches. Also, microbiological parameters such as SPC, Yeast & Mold and coliform were also scrutinized to evaluate the best result. The structure of the film was also kept in mind to examine the barrier properties such as to check the porosity and homogenous structure of the film. It could be observed that the corn starch @5 and glycerol @2 percent gave excellent result than any other starch and was chosen best. The optimized film had low thickness, moisture content & WVTR and high mechanical properties such as tearing strength & puncturing strength along with low microbial count. Also it had less porosity and heterogeneity in the structure of the film as compared to other film prepared from different starches.

5.3 Preparation and wrapping of product

Milk cake was prepared as per the procedure given by Landge *et al* 2009. The pieces of the freshly prepared milk cake were cut according to the size which can be easily consumed by consumer at the time. The film prepared for wrapping the milk cake was cut according to the sizes of the pieces of the milk cake. The piece of the milk cake was kept on the flat surface and the film was wrapped on the surface of piece in such a way so that maximum of the piece could be properly wrapped with film, replicating the wrapping of milk cake with butter paper.

5.4 Evaluating the shelf life of the product along with film at two temperature conditions

The optimized product was analyzed during storage period at two different temperatures. Various parameters like sensory and physico-chemical composition showed a gradual decline in sensory parameters during storage at ambient (25°C) and refrigeration temperature (4°C) conditions. An interval of 3 days was taken to evaluate quality of samples. The results of storage study revealed that the sensory scores of stored products gradually declined with the passage of days. The sensory scores were recorded for the color, body & texture, sweetness, flavor and overall acceptability scores of fresh milk cake with edible film and without edible film referred as control at 0 day were 8.0, 7.87, 8.2, 7.86 & 7.75 and 7.75, 7.5, 8.16, 7.8

& 7.8, respectively which gradually decreased at both refrigeration (4°C) and ambient temperature (25°C). However, the extent of decline in scores depended on the storage temperature. The changes in all the storage parameters were faster at ambient temperature (25°C) than at refrigeration temperature (4°C). However, score showed significant decrease at both temperatures. This is due to loss of freshness and fat oxidation which led to bland flavour of the product and showed bitterness. During storage, the overall acceptability depends upon many factors such as proteolysis, lipolysis and flavour changes during storage. Product was sensorily became unacceptable after 6 days at ambient storage (25°C) and acceptable after 18 days of storage at refrigeration storage (4°C). Different physico-chemical properties, like FFA of wrapped milk cake with edible film increased from 0.24 to 0.40 µeq / g and 0.24 to 0.42 µeq / g for control sample at ambient temperature (25°C) for 6 days. On the other hand, FFA of wrapped film increased from 0.24 to 0.36 µeq / g and 0.24 to 0.39 µeq / g for control sample stored at refrigeration temperature (4°C) after 18 days. The HMF value increased from 18.97 to 25.78 µ moles/100g for control sample whereas it increased from 18.38 to 25.12 µ moles/100g for wrapped sample stored at ambient temperature (25°C) after 6 days, whereas HMF value increased from 18.97 to 22.8 for control sample while it increased from 18.38 to 22.12 µ moles/100g for wrapped sample at refrigeration temperature (4°C) after 18 days. The TBA value increased from 0.237 to 0.261 for the control sample whereas it increased from 0.231 to 0.254 for wrapped sample stored at ambient temperature (25°C) after 6 day, whereas it increased from 0.237 to 0.269 for control sample and it increased from 0.231 to 0.265 OD at 532 nm for wrapped sample stored at refrigeration temperature (4°C) for 18 days. With storage at ambient (25°C) and refrigeration temperature (4°C), the tyrosine value of control sample increased significantly from 0.22 to 0.29 mg/100g in control sample whereas increased to 0.32 mg/100g for wrapped sample at ambient temperature (25°C) after a period of 6 days.

However, microbiologically, product could not stand more than 15 days and on 18 day, the Standard plate count went out of FSSAI limit for *khoa* or *khoa* based sweets. Therefore, it was considered to have a life of 15 days at refrigeration temperature (4°C).

5.5 Consumer Studies of the product with film

To get an idea about the actual consumer response of the developed product, the optimized product was given to total 110 prospective consumers of varying age group, income, education level etc. The results indicated that the product was liked by almost 96% people who participated in the study. Therefore, the technology for acceptable edible film wrapping for the milk cake has been developed successfully and being an innovative and cost-effective product, large scale production of the product can be taken up by large players of the field. The consumer survey of the product revealed that 50% consumers liked the product very much, 39% liked product extremely, 7% liked moderately and 4% liked slightly. Therefore, present investigation revealed that, milk cake wrapped with edible film is well accepted by the consumers.

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ANNEXURE –I

College of Dairy Science and Technology
Guru Angad Dev Veterinary and Animal Sciences University

Score Card for Sensory Evaluation of milk cake

Product particulars: Milk Cake with edible film **Date:**

Kindly evaluate the given sample of milk cake for colour and appearance, body & texture, sweetness, flavour and overall acceptability along with the edible film (polysaccharide-based film) using the following 9-point hedonic scale and enter the score for each sample in the space provided in the below table.

Hedonic ratings	**Score**
Liked Extremely	9
Liked Very Much	8
Liked Moderately	7
Liked Slightly	6
Neither Liked nor Disliked	5
Disliked Slightly	4
Disliked Moderately	3
Disliked Very Much	2
Disliked Extremely	1

Sensory Attributes	**Samples codes**			
	Control		Sample	
Color & Appearance				
Body & Texture				
Sweetness				
Flavour				
Overall Acceptability				

Remarks (if any**): _____**

Signature: _____

ANNEXURE – II

Performa for consumer survey of the optimized product
(Milk Cake with Edible film)
College of Dairy Science and Technology
Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana

Date: - _____

Name: - _____ Educational Status: - _____

Age: - _____ Occupation: - _____

Sex: - ☐ Male ☐ Female

Family Income: ☐ <1.5 lakh ☐ 1.5-5.0 lakh ☐ >5.0 lakh

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1. When you buy milk cake, then you give more importance to:
   - Health
   - Taste
   - Price
   - Texture of sweet
2. Did you like this sweet (milk cake)
   - Yes
   - No
3. If yes, degree of liking
   - Like extremely
   - Like very much
   - Like moderately
   - Like slightly
4. If available in market then would you like to buy this product, along with package to eat
   - Yes
   - No
5. If you will be given edible package and conventional product, would you like to eat taking into consideration
   - Hygiene practices
   - Taste of product with package
   - Maintenance of texture of good
6. Any suggestion/ remarks for this product.

   _____
   _____
   _____

_____
Signature

VITA

Name of the student	:	Navdeep Singh
Father's name	:	Gurmeet Singh
Mother's name	:	Jasveer Kaur
Nationality	:	Indian
Date of birth	:	14.06.1994
Permanent home address	:	Village Salempura, PO Sidhwan Bet, Tehsil Jagraon, Distt. Ludhiana, Punjab, 142033

EDUCATIONAL QUALIFICATION

Bachelor's degree	:	B.Tech. (Dairy Technology)
University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
Year of Award	:	2016
OCPA	:	7.17/10.00
Master's degree	:	M. Tech.
OCPA	:	7.94/10.00