EFFECTS OF PROBIOTIC AND PREBIOTIC ON NUTRITIONAL QUALITIES OF SYNBIOTIC CAKE

A THESIS

BY

NUSRAT JAHAN

EXAMINATION ROLL NO.: 1605222

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SESSION: 2016-2017

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MASTER OF SCIENCE
IN
FOOD SCIENCE AND NUTRITION

DEPARTMENT OF FOOD SCIENCE AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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ABSTRACT

The purpose of this study was to develop a synbiotic cake to which probiotic and prebiotic ingredients were added and verified the perspectives of the product with regard to potential for consumer health benefits and sensorial acceptance. Inulin was extracted and characterized for the application as prebiotic. Synbiotic cake constructed by incorporating inulin and by the application of film forming solutions (sodium alginate and glycerol) containing *Lactobacillus spp* followed by an air drying step at 60 ^{0}C for 10 min. Storage studies were performed for synbiotic cake by analyzing moisture, pH, fiber, firmness, color and microbial viability test in time interval for 0 day, 4 days and 8 days. The sensory profile of cakes with prebiotic and probiotic (synbiotic cake) and without prebiotic and probiotic (control cake) was evaluated using a nine point hedonic scale. According to our analytical results inulin obtained 5.82% moisture, 0.58% ash, 5.52 pH, 0.04 mg/ml total sugar, 0.07 mg/ml reducing sugar, 0.03 mg/ml non reducing sugar along with the comparable functional properties (71.64 g/l solubility, 2.16 g of water/g of sample water absorption capacity, 1.57 ml of water/g of sample swelling capacity). The results revealed that moisture content, pH sample fiber content and firmness of all prepared sample (control sample to T_3S_5) ranged from 11.56 to 18.68 %, 9.79 to 10.02, 2.80 to 4.34 % and 2.19 to 2.95 N respectively. Probiotic populations varied from 7.767 up to 7.823 log cfu g^{-1} (Before *In vitro*) and from 6.71 up to 6.79 log cfu g^{-1} (after digestion).Significant difference ($P<0.05$) between the control and synbiotic cakes was observed in case of moisture, pH, fiber, firmness, color and viability test of probiotic. All samples showed a significant ($P<0.05$) change for P^H, firmness and color while there was no significant change in moisture, fiber and viability count during 8 days of storage. Sensory evaluation of cakes indicated that all samples were in acceptable limit. The T_2S_3 (2% inulin+ 50:50 film forming solution) sample scored best in terms of sensory attributes among the sample though T_3S_3 (3% inulin+ 50:50 film forming solution) obtained best result for fiber, firmness and microbial count (before and after in- vitro digestion). To be concluded T_2S_3 can be considered as the best sample in terms of nutritional and sensory attributes.

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LIST 0F ABBREVIATION

AOAC	Association of Analytical Chemists
FAO	Food and agriculture Organization
HDPE	High Density Poly Ethylene
WHO	World Health Organization
WAC	Water Absorption Capacity
PBS	Phosphate Buffer Saline
ANOVA	Analysis of variance
CFU	Colony Forming Unit
BHT	Butylated Hydroxy Toluene
MRS	Man, Rogosa and Sharp
DNS	3, 5–di-nitro salicylic acid
L.S.D	Least significant difference
SE	Standard error
et al.	and other
°C	Degree centigrade
min	Minute
SE	Standard error
spp.	Species
mL	milliliter
%	Percentage
g	Gram
Fig.	Figure
g/ L	gram per liter

CHAPTER I

INTRODUCTION

Functional foods are those that provide basic nutrition and at the same time, promote health (Halsted, 2003). As the market for these products continues to expand, research in the development of food products containing probiotic bacteria will also continue to grow (Boylston *et al.*, 2004). Actually, there are two dietary strategies to increase the beneficial microorganisms of the gut microbiota. The first one is by the consumption of probiotics and the other one is by increasing the number of resident microorganisms in the gut tract, using prebiotics (Ferreira & Teshima, 2000).

Probiotics are live microorganisms that when ingested in adequate amounts confer a health benefit on the host (FAO/ WHO, 2006). Probiotics are distincted as selected, viable microbial food supplement, when introduced in sufficient quantities beneficially affect the host by improving the microbial balance in the intestinal tract (Zimmer & Gibson, 1998; Sanders, 1998; Vaughan *et al.*, 1999; Zubillaga *et al.*, 2001; Holzapfel & Schillinger, 2002). The most commonly-used and best-studied probiotic organisms are the bacteria *viz Lactobacillus spp*, *Enterococcus* spp, *Bifidobacterium*, and the yeast *viz. Saccharomyces boulardii*. *Lactobacillus spp. (L. acidophilus)* is the most commonly used probiotic or beneficial bacteria. The application of the probiotic in food sector has been incredible over the last decade with dairy products i.e. yogurt, ice cream, cheese and milk, juices and beverages and infant formulations being predominant (Euromonitor, 2012).

Probiotic has a crucial role in the development of numerous conditions and some of the beneficial effects of probiotic consumption include reducing obesity (Clarke *et al.*, 2013; Ley *et al.*, 2006), helping fiber digestion (Morrison & Preston, 2016), preventing viral, bacterial & fungal infections (Gregor & Jeremy, 2002), helping brain health development (Cryan & Dinan, 2012)**,** improving blood pressure & cholesterol level (Upadrasta and Madempudi, 2016; Lay & Min, 2010), stimulating and developing of the immune system (Rooks & Garrett, 2016; Levy *et al.*, 2017), preventing inflammatory bowel diseases (Quigley, 2012; Strober *et al.*, 2008), reducing symptoms of lactose intolerance (de Vrese *et al.*, 2001). Probiotic can be used in food directly. But probiotic viability in foods depends on various factors during processing and storage because processing and storage

conditions for instance thermal processing are the main obstacles to food manufacturers (Bustos & Borquez, 2013). Biodegradable films or coatings may improve the viability of probiotics during heat processing (Kanmani & Lim, 2013).

Addition of prebiotics can enhance the efficiency of probiotics. Prebiotics are nonviable food components that assist both beneficial bacteria which are already established in the colon as well as externally administered probiotic bacteria by stimulating the growth or activity (FAO/WHO, 2006). Prebiotics are short chain carbohydrates which are not digested or poorly digested by digestive enzymes in humans and pass by the small intestine to the lower gut and become accessible for probiotic bacteria without being utilized by other intestinal bacteria (Al-Sheraji *et al.,* 2013). The most popular prebiotics are - fructooligosaccharides (FOS) and inulin, galactooligosaccharides (GOS), polydextrose, resistant starch, soyoligosaccharides, xylo-oligosaccharides, isomaltooligosaccharides, and lactulose (Gibson *et al.*, 2004). Among prebiotics, inulin have been researched a lot as a food supplement (Vanloo *et al.*, 1995) and exerted a protective effect on lactic acid bacteria (LABs) by improving their survival and activity during storage of the final product (Gustaw *et al.*, 2011). Besides acting as a prebiotic (Kelly, 2008, 2009; Kolida *et al.*, 2007), inulin helps regulating blood sugar, increasing bone absorption of calcium (Lobo *et al.*, 2009), raising resistance to gastrointestinal infections (Sauer *et al.*, 2007; Wendy *et al.*, 2008), helping prevent arterial hypertension (Rault - Nania *et al.*, 2008) and cancer of the colon (Gibson *et al.*, 2005; Sauer *et al.*, 2007; Davis & Milner, 2009).

A synbiotic is a supplement containing both prebiotic (the food components they live on) and probiotic (the live bacteria) that work together in a synergistic way i.e. the number and activity of beneficial intestinal microbiota is increased through the use of prebiotic (Panesar *et al.*, 2009; Gibson *et al.*, 2004). Roberfroid (2000) suggested that these products can improve the survival of bacteria when they pass into the upper part of the gastrointestinal tract, and produce greater effects in the large bowel.

The viability of bacterial cells after consumption remains obscure as the bacteria are also subjected to unfavorable physiological conditions of the gastrointestinal (GI) tract such as acidic environment and bile secretions (Holzapfel *et al.*, 1998). Viability of these bacteria upon ingestion and sufficient survival through the transit to GI tract is crucial to confer any health benefits to the host (Hou *et al.*, 2003, Kailasapathy, 2006). At present

In vitro digestion models gain more attention of the researchers in different food systems (Toor *et al.,* 2009, Wootton-Beard *et al.,* 2011). *In vitro* digestion is a rapid and inexpensive method used to determine the availability of nutrients as well as bacterial viability in the small intestine (Prajapati *et al.*, 2013).

In last decades processed foods like fast food, baked and confectionary products are getting more popularity because of its convenience, attractive appearance, taste and texture. Among bakery products consumed, cakes are particularly popular and associated in the consumer mind as a delicious product (Zhang *et al.*, 2012). On the contrary synbiotic products have not been intensively studied (Bielecka *et al.,* 2002). To the best of our knowledge data about synbiotic cake are not existent and the development of such a novel functional baked product combining probiotic and prebiotic ingredients may be a good alternative of healthy food. On the basis of this background, there is a great importance of studing the effect of inulin and the synbiotic interaction between probiotic deserve to be explored for the production of new baked product with improved quality.

Thus the specific objectives of this study were:

1. To extract and characterize the inulin from garlic and culture the *Lactobacillus spp.*
2. To evaluate the nutritional composition and sensory evaluation of prepared synbiotic cake.
3. To explore the effect of *In vitro* digestion on viable count.
4. To assess the storage stability of the synbiotic cake.

CHAPTER II

REVIEW LITERATURE

2.1 Prebiotic

2.1.1 General introduction on prebiotic

In 1995, Gibson and Roberfroid introduced the prebiotic concept. Prebiotics are short-chain carbohydrates (SCCs) that are non-digestible by digestive enzymes in the small intestine (Quigley *et al.*, 1999). They are sometimes referred to as non-digestible oligo - saccharides (NDOs) which are soluble in 80% ethanol. Prebiotic is a non active food constituent that shifts to the colon and is then selectively fermented. The benefit to the host is mediated during selective stimulation of the growth and/or activity of one or a limited number of bacteria (Gibson & Roberfroid, 1995).

According to the FAO (2008), prebiotics are "nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria". Recently, scientists defined a dietary prebiotic as ''a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health'' (Gibson *et al.*, 2010). Prebiotics are not broken down by gastric enzymes, but pass unaltered into the large intestine, where they are then selectively fermented, producing beneficial effects, such as production of short chain fatty acids (Williams NT, 2010). Dietary fiber is the most commonly utilized prebiotics (Gibson & Roberfroid, 1995).

Lactulose, galactooligosaccharides, fructooligosaccharides, inulin and its hydrolysates, maltooligosaccharides, and resistant starch are usually used prebiotics in the human diet. The essential end components of carbohydrate metabolism are short-chain fatty acids, particularly acetic acid, propionic acid and butyric acid, which are used by the host organism as an energy source. They can also be found in different sources such as chicory, onion, garlic, asparagus, artichoke, leek, bananas, tomatoes and many other plants. Generally, oligosaccharides are combinations of sugars with a different degree of polymerization (Crittenden & Playne, 1996). Prebiotic oligosaccharides can be manufactured by three different methods: isolation from plant resources, microbiological production or enzymatic synthesis, and enzymatic degradation of polysaccharides

(Crittenden & Playne, 1996; Gulewicz *et al.*, 2003). Most of prebiotic oligosaccharides are manufactured and are generally available in the markets. A large number of patents regarding prebiotic oligosaccharides have been filed and their number is growing (Grajek *et al.*, 2005).

These prebiotic has some criteria such as: 1) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption; 2) fermentation by intestinal microflora; and 3) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being (Gibson *et al.*, 2004).

2.1.2 Health benefits of prebiotics

Prebiotics have been suggested of having various health benefits on humans. It acts as a substrate for the useful gut microorganisms. The various benefits are:

- **Promotion of Normal Colon Transit Time:** Constipation is an exceedingly common clinical problem affecting large segments of the population including the elderly, pregnant and nursing women, people on weight loss diets, and people with disrupted daily schedules such as variable shift workers and business travelers (Kaur and Gupta, 2002; Brandt LA, 2001). Prebiotics increase fecal bulk and optimize stool consistency primarily by increasing fecal microbial mass. This increase in fecal bulk stimulates passage through the colon, shortening transit time. Colonic water resorption is reduced, stool becomes softer and heavier, and stool frequency increases. Together these factors alleviate constipation and improve colon evacuation. In a study of constipated elderly adults, 20 grams per day of inulin-type fructans had a significantly better laxative effect than lactose (Kleessen *et al.*, 1997). A mixture of inulin-type fructans and galactooligosaccharides has been repeatedly shown to improve the stool frequency and consistency of bottle-fed infants similar to that of breast-fed infants (Moro *et al.*, 2002). Administration of isomalto - oligosaccharides has been shown to increase stool frequency and wet stool output in constipated elderly men (Chen *et al.*, 2001). Xylooligosaccharides have been shown to reduce severe constipation in pregnant woman (Tateyama *et al.*, 2005) and lactulose administration has a long clinical history of alleviating constipation (Schumann C, 2002).

- **Production of Short-Chain Fatty Acids:** Prebiotics are primarily energy sources for healthful intestinal bacteria that ferment them into short-chain fatty acids**.** Many of the benefits of prebiotics derive from increased bacterial production of short chain fatty acids. Much of the increase in short-chain fatty acids comes about through metabolic cross-feeding in which prebiotics are fermented by certain species, such as Bifidobacterium, into end products that are in turn metabolized by other microorganisms resulting in an increased quantity and diversity of short-chain fatty acids (Belenguer *et al.*, 2006; Flint *et al.*,2007)**.** Acetate is usually the dominant short-chain fatty acid in the colon followed by approximately equal concentrations of propionate and butyrate (Cummings *et al.*, 1987)**.** Short-chain fatty acids play essential roles in the growth and physiology of intestinal tissue as well as in systemic metabolism (Topping and Clifton, 2001; Saemann *et al.*, 2002)**.**

- **Inflammatory bowel disease**: Inflammatory bowel disease has been related to the intestinal microbiota pathogenesis. One of the understandable ways for therapeutic intervention is probiotic treatment. A mixture of long chain inulin and oligosaccharide has shown the ability to decrease the inflammatory histological and gross cecal scores in the cecum and colon. It also decreased the levels of the pro-inflammatory cytokine IL-1β and increased the anti-inflammatory TGF-β, at the same time it increases cecal lactobacillus and bifidobacterium levels (Hoentjen *et al.*, 2003).

- **Effects of prebiotics on bone mineralization**: Bone mass of an adult is dependent on supply as well as bioavailability of calcium. In either cases of deficiency osteoporosis is prone to occur and in addition to this it is highly associated with increasing age and postmenopausal conditions. Presently, osteoporosis treatment and prevention is restricted to increasing calcium uptake, or by stimulating bone formation. Even though, a lot of studies have been carried out on calcium metabolism using rats, results depicted that prebiotics play a role in escalating the bioavailability of calcium. But so far, only a few human trials have been translated from these results. Prebiotic consumption increasing calcium absorption has the majority supporting data from the rat studies where as human trials have had diverse results (Wong *et al.*, 1989).

- **Influences on Glucose & Insulin Levels:** Evidence suggests prebiotics can favorably influence serum glucose and insulin levels in a variety of ways. DGOs and other prebiotics can reduce the amount of glucose available for absorption into the bloodstream. Prebiotics also prevent excessive blood glucose elevations after a meal by delaying gastric emptying and/or shortening small intestine transit time. Bacterial fermentation yielding short-chain fatty acids is another mechanism whereby prebiotics can modulate glycemia and insulinemia. Propionate has been shown to reduce hepatic gluconeogenesis and enhance hepatic glycolysis (Roberfroid and Delzenne, 1998) and fermentation end products, mainly butyrate, are believed to be responsible for increases in the glucose-regulating and satiety-inducing hormone glucagon-like peptide-1 (GLP-1) observed in prebiotic-fed animals (Delzenne *et al.*, 2007). Consumption of 20 grams/day of inulin-type fructans by healthy volunteers did not modify fasting plasma glucose and insulin concentrations, but decreased basal hepatic glucose production after 4 weeks. Another study examining the effects of 10 grams/day of inulin in healthy middle-aged men and women reported significantly decreased insulin concentrations after 4 weeks (Jackson *et al.*, 1999). A study of non-insulin-dependent diabetic subjects administered 8 grams/day of inulin-type fructans reported significantly lower blood glucose levels after 4 weeks (Yamashita *et al.*, 1984).

2.2 Inulin as a prebiotic

2.2.1 Introduction

Inulin was discovered over two centuries ago by Rose (Fluckiger & Hanbury, 1879) and since then its presence in many plants became apparent (Livingston *et al.*, 2007). Some examples of plants containing large quantities of inulin are Jerusalem artichoke, chicory root, garlic, asparagus root, salisfy and dandelion root (Kaur & Gupta, 2002). More commonly consumed vegetables and fruits containing inulin are onion, leek, garlic, banana, wheat, rye and barley. Over the past decades, a lot of research has been done showing that inulin is a versatile substance with numerous promising applications. Several reviews have been published on inulin, its characteristics and functionality in food (Boeckner *et al.*, 2001; Kelly, 2008, 2009; Seifert & Watzl, 2007) and pharma (Imran *et al.*, 2012).

Inulin could act as a substitute for sugar or fat, having the advantage of very low caloric value. Also, presents some functional properties. It acts in the organism in a similar way to dietary fibers, contributing to the improvement of the gastrointestinal system conditions. It was observed that inulin increase viscosity, giving ‘‘body’’ and optimizing the texture of low-calorie beverages as well as providing spread-ability to low fat and no fat products, yogurts, salad dressings, mousses, chocolates, etc. (Gibson *et al.*, 2004).

2.2.2 Uses

Inulin is widely applied in the food industry and it serves many purposes. It has been used as a (low calorie) sweetener, to form gels, to increase viscosity, to improve organoleptic properties, and as anon-digestible fiber. Mostly it is used as a sugar and fat replacer in dairy products and as a prebiotic (Meyer *et al.*, 2011). Examples of use in dairy are application in cheese, milk, yogurt and ice cream (Meyer *et al.*, 2011). Some examples of use of inulin in non-dairy food are use in bread, biscuits, cereal and meat products (González-Herrera *et al.*, 2015; Karimi *et al.*, 2015; Kuntz *et al.*, 2013; Furlán *et al.*, 2015). Previous reports have already extensively reviewed the food applications of inulin as a prebiotic (Kelly, 2008, 2009; Kolida, Tuohy, & Gibson, 2007; Roberfroid & Delzenne, 1998).

Applications of inulin as pharmaceutical excipient are even more diverse and range from stabilization of protein-based pharmaceuticals (Hinrichs *et al.*, 2001), through solid dispersions to increase dissolution rate (Visser *et al.*, 2010), to targeted colon delivery. (Imran *et al.*, 2012). Moreover, as mentioned earlier, inulin itself is used as a diagnostic tool for measuring the kidney function (glomerular filtration rate) (Orlando *et al.*, 1998). Inulin is injected intravenously, after which it is excreted renally. As inulin is not naturally present in the body and it is not metabolized in circulation, the amount of inulin secreted in the urine provides information on kidney function. Less widespread is the use of inulin for industrial and chemical purposes. Stevens *et al.*, (2001) reviewed the derivatization of inulin and applications of these chemically modified inulins for a wide range of applications, from inhibiting calcium carbonate crystallization industrially to use in hair gel.

Inulin is used in food as a texture modifier and fat replacer because of its DP-dependent gel forming and viscous behavior. The (2→1) glycosidic bonds of inulin make it indigestible to humans and it can therefore be used as a low-calorie sweetener, fat

replacer and dietary fiber (Barclay *et al.*, 2010). Colonic microorganisms such as *Lactobacilli*, however, are capable of breaking down this bond, making inulin suitable for colonic targeting. The relatively high glass transition temperature of amorphous inulin in combination with its flexible backbone makes it a good stabilizer of proteins applied both pharmaceutically (Tonnis *et al.*, 2015) and in food (Furlán *et al.*, 2012). Lastly, specific crystalline morphologies make inulin suitable as an adjuvant for vaccines (Honda-Okubo *et al.*, 2012).

2.2.3 Stability of inulin

When prebiotics are added to processed foods, stability during processing becomes an important factor. Processing conditions such as heat, acidity, and Maillard reactions may have an effect on the stability of prebiotics (Huebner *et al.*, 2008). Several studies have been completed to determine the chemical stability of prebiotics when exposed to certain processing conditions. The stability of a prebiotic varies based on its properties. Inulin are susceptible to acid hydrolysis; Polydextrose is stable, but can be degraded with extreme conditions (Beer *et al.*, 1991; Blecker *et al.*, 2002; Courtin *et al.*, 2009; Klewicki, 2007; Playne and Crittenden, 1996).

Inulin were susceptible to acid hydrolysis when exposed to a low pH (3) and a moderate temperature (70°C) (Blecker *et al.*, 2002). When released fructose molecules were monitored, there was an increase of fructose when chain lengths were shorter, thus FOS was more rapidly hydrolyzed than inulin. Inulin was hydrolyzed at a slower rate to begin and increased as the chain length was decreased (Blecker *et al.*, 2002). Similar studies have concluded acid hydrolysis has occurred for FOS (Courtin *et al.*, 2009; L'Homme *et al.*, 2003) .

When exposed to extreme dry heat (195°C), inulin was degraded substantially, to a degree of polymerization of less than 5, after 30 minutes (Bohm *et al.*, 2005). Inulin may also encounter hydrolysis from inulinases. This hydrolysis was increased with an increase in temperature to a point, but once the temperature reaches a certain level, around 50°C, the inulinases started to denature (Cantana *et al.*, 2007).

When the prebiotic is chemically modified or degraded, there is a possibility that the degraded fractions of the prebiotic still provide biological activity in the large intestine (Huebner *et al.*, 2008). The biological activity of inulin was determined using an *In vitro*

batch method for samples that were exposed to heat, acidity, and Maillard reaction conditions (Huebner *et al.*, 2008). The effect of low pH (3) alone did not change the prebiotic activity, but when combined with heat (85°C for 30 minutes), loss of activity was observed for inulin. The Maillard reaction conditions did not significantly affect the prebiotic activity for inulin (Huebner *et al.*, 2008).

2.3 Probiotics

2.3.1 General introduction

The term "probiotic" originates from the Greek word meaning "for life" (Fuller, R, 1989). The first clinical trials were performed in the 1930s on the effect of probiotics on constipation. The discovery by Mann and Spoerig (1974) that people who drank yogurt fermented with wild strains of *Lactobacillus sp.* had very low values for blood serum cholesterol opened up a new area of study. Harrison *et al.* 1975 reported that cells of *Lactobacillus acidophilus* added to infant formula decreased levels of serum cholesterol, and Gill and Guarner (2004), showed control of serum cholesterol levels in adult human experiments. In 1994, the World Health Organization deemed probiotics to be the next-most important immune defence system when commonly prescribed antibiotics are rendered useless by antibiotic resistance (Kailasapathy and Chin 2000; Levy 2000). The use of probiotics in antibiotic resistance is termed as a microbial interference therapy.

In 2004, Gibson *et al.* defined prebiotics, on the other hand, as "a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" The majority of probiotic bacteria are gram positive and they produce lactic acid. The genera of *Lactobacillus* and *Bifidobacterium* compose most of members of probiotic bacteria. *Saccharomyces boulardii (S. boulardii), Saccharomyces cerevisiae (S. cerevisiae), Escherichia coli (E. coli) and Bacillus spp.* are also included in some probiotic recipes (De Vrese & Schrezenmeir 2008).

2.3.2 Health benefits of probiotics

➢ **Inhibition of pathogenic microorganisms:**

Probiotics prevent colonization of pathogenic microorganisms by producing antimicrobial peptides and compounds such as bacteriosin, organic acids and hydrogen peroxide. Production of organic acids by probiotic bacteria lowers the intestinal pH which then inhibits the growth of pathogenic microorganisms (Ng *et al.* 2009). For instance, *Salmonella typhimurium (S.* typhimurium) which causes Salmonellosis is unable to survive acidic conditions (Salminen & Wright 1998). Corr *et al.* (2007) showed that *Lactobacillus salivarius* protected mice from *Listeria monocytogenes (L. monocytogenes)* infection through the direct action of bacteriosin Abp118. In addition, sakacin produced by *Lactobacillus sakei (L. sakei)* 1 was also shown to inhibit the adherence of *L. monocytogenes* on stainless steel surface (Winkelströter *et al.* 2011). Probiotics also inhibit the growth of pathogens through competition for micronutrients and attachment site on the intestinal epithelia. Attachment of probiotics on the epithelial cell surface blocks the binding of pathogenic microorganisms (Kopp-Hoolihan 2001). The effectiveness of probiotics in inhibiting the growth of pathogenic bacteria has led to the use of probiotics in treating diarrhea and preventing pathogen infection in the intestine. For example, prevention of antibiotic associated diarrhea. A number of clinical studies demonstrated that ingestion of *S. boulardii, Lactobacillus rhamnosus GG (L. rhamnosus GG), L. acidophilus, L. bulgaris* reduced the occurrence of antibiotic associated diarrhea by as much as 52% (Sazawal *et al.* 2006).

➢ **Reduction of lactoce intolerance:**

Lack of β-galactosidase activity in the lower intestine causes lactose intolerance (Vasiljević & Shah 2012). The beneficial effects of probiotics on lactose intolerance are explained by two ways. One of them is lower lactose concentration in the fermented foods due to the high lactase activity of bacterial preparations used in the production. The other one is; increased lactase active lactase enzyme enters the small intestine with the fermented product or with the viable probioticbacteria (Salminen, *et al.* 2004). When the yogurt is compared with milk, cause the lactose is converted to lactic acid and the yogurt consist of bacterial β-galactosidase enzyme; it is suitable end beneficial to

consume by lactose intolerant. Furthermore, the LAB which is used to produce yogurt, *Lactobacillus bulgaricus and Streptococcus thermophilus*, are not resistant to gastric acidity.

- **Enhancement of host immune function:**

Probiotics regulate host immune system by activating nuclear factor kappa beta (NF-κB), balancing T-helper cell response, stimulating the production of IgA, controlling inflammatory reactions and increasing the activity of macrophages (Kalliomaki & Walker 2005). However, a balanced inflammatory response is relatively important in order to avoid excessive intestinal inflammation which leads to severe intestinal disorders such as infl amatory bowel disease (IBD) and necrotizing enterocolitis (NEC).Intestinal lymphoid tissues are stimulated by probiotic bacteria attachment to Toll-like receptors (TLRs) expressed on macrophages, dendritic cells, B cells and epithelial cells (Pasare & Medzhitov 2005). Such attachment induces the production of cytokines by immune cells and secretion of polymeric IgA by plasma cells (Kohler *et al.* 2003). Bacterial ligands on the surface of probiotic bacteria such as lipopolysaccharide (LPS) and lipoteichoic acid interact with TLR-2 and TLR-4 of dendritic cells and subsequently activate NF-κB which then migrates to the cell nucleus and binds to cytokine promoters. This binding then initiates infl ammatory cytokine mRNA transcription. Pro- inflammatory cytokines such as TNF-α (tumor necrosis factors), IL (interluekin)-1β, IL-8 and IL-6 are produced and subsequently involved in the activation of naïve T helper cells (Th0) into T helper (Th)1, Th2 and T-regulatory (Treg) cells (Momoko 2005). Th1 immune response is important in eliminating intracellular pathogens while Th2 response protects hosts against parasitic and extracellular pathogen infections (Delcenserie *et al.* 2008).

- **Crohn's disease and ulcerative colitis:**

Crohn's disease (CD) and ulcerative colitis (UC) are the chronic diseases of GIT with more or less common symptoms. Both are collectively called inflammatory bowel disease (IBD). In ulcerative colitis only mucosa and sub mucosa of colon are inflamed. In case of Crohn's disease, the mucosa, submucosa and serosa are inflamed and the inflammation can spread to whole GIT. Crohn's disease is associated with diarrhea, weight loss and abdominal pain while Ulcerative colitis has the symptoms of diarrhea and bleeding (Jonkers and Stockbrügger, 2003). Exact cause of Inflammatory bowel

disease in not known. Probiotics are used in the treatment of inflammatory bowel disease considering that bacteria are involved in the etiology of the disease. Different studies show beneficial effect of probiotic in the treatment of inflammatory bowel disease in animal models (Gionchetti *et al.*, 2002). Clinical placebo controlled studies also shows that probiotics cause improvement in the condition of IBD (Jonkers and Stockbrügger 2003).

- **Colon cancer:**

Management of cancer is one of the hot issues in these days (Ilayas and Qadir, 2010; Tabasum and Qadir, 2010; Bokhari *et al.*, 2012; Farooqi *et al.*, 2013; Saleem *et al.*, 2013). In laboratory experimentation, some stains of LAB *(Lactobacillus delbrueckii)* have shown anti-mutagenic effects because they have ability to bind with heterocyclic amines which are carcinogenic (Wollowski *et al.*, 2001). Animal studies proved beneficial effects of LAB against colon cancer of rodents. Human trials also suggest that some types of LAB are anti-carcinogenic due to ability to decrease the activity of enzyme called β glucuronidase (Brady *et al.*, 2000) (which can generate cancer producing substances in the digestive system). The incidence of colon cancer in people consuming dairy product has been low compared to others during population studies. But there is still lot more to do to confirm this effect.

- **Liver diseases:**

The liver and gut has an important relation in a sense that the blood is carried form gut to the portal system. Liver functions are stimulated by intestinal blood content. Similarly bile secretion produced by liver affects gut performance. It is found that any change in the normal composition of gut micro flora alter liver function and can lead to initiation and progression of liver diseases (Cesaro *et al.*, 2011). Many complications (Hepatic ence- phalopathy, cirrhosis, spontaneous bacterial peritonitis) are associated with overgrowth of harmful bacteria, changed intestinal permeability and improper immune function. Probiotics are useful in the treatment of chronic liver diseases as they block entry of microorganisms to blood flow and ultimately to liver by increasing the strength of intestinal barrier (Cesaro *et al.*, 2011). Another mechanism involves the regulation of gut micro flora (Sheth and Garcia, 2008) and regulation of immune functions (Jonkers and Stockbrügger, 2007). These also reduce the development of Hepatic encephalopathy

and also support other medication for the treatment of Hepatic encephalopathy (Hopkins, 2003).

- **Dental caries:**

One of the major causes of dental caries is mutant streptococci. It is suggested that the products containing probiotics when used, can decrease the number of mutant strain of streptococci (Nase *et al.*, 2001; Cildir *et al.*, 2009; Haukioja, 2010). In these studies it is found that consumption of food containing probiotics also enhances the level of slivary probiotics (Ahola *et al.*, 2002; Montalto *et al.,* 2006).

- **Oral health:**

Ptobiotics reside in oral cavity in a very less numbers, comprising only 1% of total microbiota of oral cavity (Haukioja, 2010). Probiotic species present in sliva includes *L. paracasei, L. plantarum, L. salivarius, and L. rhamnosus (Haukioja, 2010; Ahrne et al., 1998)*. Bifidobacterial species which are found to be residing in oral cavity are *B. bifidum, B. dentium, and B. longum* (Haukioja, 2010). Probiotics enhance dental care by 3 possible mechanisms which include modulation of immune response, Normalization of oral microflora and metabolic effects (Parvez *et al.*, 2006). Probiotics improve oral health by inhibiting incidence of following diseases.

- **Food allergy:**

Food allergy is caused by the antigens present in food and is associated with inflammation of intestine. Probiotic are helpful in reducing the symptoms of food allergy as they enhance gut defence by two mechanisms (nonimmunologic and immunologic). First is carried out by normalizing the gut microflora and decreasing membrane permeability. Second mechanism involves the enhancement of immunological defense system of host by boosting the IgA action. This leads to enhanced degradation of food antigens and food allergy is reduced (Kirjavainen *et al.*, 2001).

2.4 *Lactobacillus spp.* as a probiotic

2.4.1 Introduction

In 1901 Beijerinck proposed the Genus *Lactobacillus*. *Lactobacilli* are Gram-positive, non-spore forming rods or coco bacilli with a G+C content of DNA below 50%. They

are catalase- negative and grow well under anaerobic conditions, but an enhanced growth is often found at reduced oxygen pressure, i.e. under micro aerophilic conditions. They prefer slightly acidic conditions (pH 5.5 to 6.5). Lactobacilli are strictly fermentative and have complex nutritional requirements (e.g. for carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivates, and vitamins) (Beijerinck, 1901).

Lactobacilli have long been the most prominent probiotic microorganisms because of their association with popular fermented dairy products. *Lactobacilli* are Gram- positive rods and part of the large group of lactic acid producing bacteria. Human strains of lactobacilli usually are part of the normal microflora of mouth, lower small intestine, colon and vagina. Fermentation of carbohydrates by lactobacilli produces lactic acid, so it survives well in acidic environments like stomach. They are rarely pathogenic (Ammor *et al.* 2007).

They produce lactic acid as the sole or one of the main products of metabolism and some species also produce acetate. Therefore, these organisms are acid uric and are able to produce a pH of four in medium or food containing fermentable carbohydrate. Creating this acidic environment allows the *Lactobacilli* to inhibit the growth of other bacteria. Several species of *Lactobacillis* have also demonstrated the ability to produce other inhibitory substances, including hydrogen peroxide, diacetyl, reuterin and bacteriocins (Ouwehand *et al.*, 2002; Stiles and Holzapfel, 1997).

The probiotic effects of lactobacilli in humans are fully documented and are strain- and dose-dependent. New prospects include probiotic therapy (Gill & Guarner, 2004), involving the use of higher doses. Clinical trials have often investigated the effects of ingesting capsules containing large amounts of probiotic (10^9–10^{10} CFU per capsule) (Kuisma *et al.*, 2003; Kajander *et al.*, 2005).

Several reviews have documented the potential of lactobacilli to promote general health: alleviation of lactose intolerance (Levri *et al.*, 2005), positive effects on the intestinal flora (Qin *et al.*, 2005), prevention of intestinal tract infections (Macfarlane & Cummings, 2002; Reid & Burton, 2002), stimulation of the immune system (Cross, 2002), reduction of inflammatory or allergic reactions (Bongaerts & Severijnen, 2005; Viljanen *et al.*, 2005), regulation of gut motility (Hamilton-Miller, 2004), and promotion of a feeling of well-being (Torriani & Marzotto, 2003). Specific health effects have also been reported: prevention of colon cancer (Wollowski *et al.*, 2001; Roller *et al.*, 2004;

Floch, 2005), decreases in blood lipid concentration and heart disease (Usman & Hosono, 2001; Liong & Shah, 2005), antihypertensive effects (Salminen *et al.*, 2004), protection against urogenital infections (Marelli *et al.*, 2004; Merk *et al.*, 2005) and Helicobacter pylori (Hamilton-Miller, 2003), and antidiabetic effects (Tabuchi *et al.*, 2003).

2.4.2 Application of Lactobacillus in food sector

Lactobacilli are widely applied in the food industry as the acids they produce suppress pH below the growth range causing metabolic inhibition of most pathogenic bacteria (Gruger & Gruger, 1989).

Lactobacillus spp have been traditionally used as starter cultures for the fermentation of food and beverages, as a cause of their contribution to flavour, aroma development and spoilage retardation. Fermented foods and drinks are now produced in a wide variety of raw agricultural materials, including e.g. milk, meat, fruits and vegetables, cereals (Wood, 1985; Buckenhüskes, 1993). These fermented foods and beverages have become a significant part of the food processing industry. Lactobacilli are important in the production of foods that require lactic acid fermentation, notably for the production of cheeses (*Lb. delbrueckii ssp., Lb. helveticus, Lb. casei*), buttermilk (*Lb. delbrueckii*), fermented milk (*Lb. acidophilus*), Kefir (*Lb. delbrueckii*) and yoghurt (*Lb. delbrueckii*). *Lactobacilli* are further employed in the production of sausages (*Lb. casei, Lb. curvatus, Lb. plantarum*), various breads (*Lb. sanfrancisco, Lb. brevis*) and fermented vegetables (*Lb. plantarum*).

They are also applied in wine industry both for grape and fruit wines, such as cider. The organic acids existing in wine which are mainly malic and tartaric acid can be easily metabolised by *Lactobacilli* (Board, 1983). Malic acid is converted to lactic acid and carbon dioxide, this phenomenon is called malolactic fermentation which is extensively used for fruit wines maturation (Liu *et al.*, 2003).

2.5 Edible film

2.5.1 Background

The greatest hurdle of the food industry is the limited shelf life of food products, a consequence of oxidation reactions such as degradation, enzymatic browning, and

oxidative rancidity (Fortuny and Belloso, 2003). One approach to reduce food deterioration is to use edible films and coatings.

An edible film is defined as a thin layer, which can be consumed, coated on a food or placed as barrier between the food and the surrounding environment. For the past 10 years, research on edible films and coatings in foods is driven by food engineers due to the high demand of consumers for longer shelf–life and better quality of fresh foods as well as of environmentally friendly packagings (Tharanathan, R. N., 2003; Cha, D. S.; Chinnan and M. S., 2004; Siracusa *et al.*, 2008). The most familiar example of edible packaging is sausage meat in casing that is not removed for cooking and eating. Such films can mechanically protect foods, prevent the contamination from microorganisms, prevent quality loss of foods due to mass transfer (e.g. moisture, gases, flavours, etc. Indeed, edible films and coatings can be used as a vehicle for incorporating natural or chemical antimicrobial agents, antioxidants, enzymes or functional ingredients such as probiotics, minerals and vitamins (Bifani *et al.*, 2007; Vargas *et al.*, 2008).

The films can enhance the organoleptic properties of packaged foods provided that various components (such as flavorings, colorings and sweeteners) are used. The films can be used for individual packaging of small portions of food, particularly products that are currently not individually packaged for practical reasons. These include pears, beans, nuts and strawberries. In a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface. Another possible application for edible films could be their use in multilayer food packaging materials together with non edible films. In this case the edible films would be the internal layers in direct contact with food materials (Murray and Luft, 1973; Kester and Fennema, 1986; Nelson and Fennema, 1991). Natural polymers or polymers derived from natural products, like food protein, offer the greatest opportunities since their biodegradability and environmental compatibility are assured (Krochta and De Mulder-Johnston, 1997). In addition, films made from protein can supplement the nutritional value of the food (Gennadios and Weller, 1990). The mechanical properties of protein-based edible films are also better than those of polysaccharide and fat based films because proteins have a unique structure (based on 20 different monomers) which confers a wider range of functional properties, especially a high intermolecular binding potential (Cuq *et al.*, 1995). Protein-based edible films can form bonds at different positions and offer high potential for forming numerous linkages. However, the poor water vapor resistance of

protein films and their lower mechanical strength in comparison with synthetic polymers limit their application in food packaging (Ou *et al.*, 2005).

2.5.2 Sodium alginate as an edible film

Alginate is of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing (King, 1983; Moe *et al.*, 1995). Sodium Alginate is a natural polysaccharide product extracted from brown seaweed that grows in cold water regions. It is a hydrophilic colloidal carbohydrate extracted with dilute alkali from various species of brown seaweeds (Phaeophyceae). In molecular terms, it is a family of unbranched binary copolymers of (1-4) - linked b-d-mannuronic acid and a-l-guluronic acid residues of widely varying composition and sequential structure (King, 1983; Moe *et al.*, 1995). Alginic acid is the only polysaccharide, which naturally contains carboxyl groups in each constituent residue, and possesses various abilities for functional materials (Ikeda *et al.*, 2000). The most useful and unique property of alginates is their ability to react with polyvalent metal cations, specifically calcium ions, to produce strong gels or insoluble polymers (Grant *et al.*, 1973; King, 1983).

Edible films prepared from alginate form strong films and exhibit poor water resistance because of their hydrophilic nature (Borchard *et al.*, 2005). The water permeability and mechanical attributes can be considered as moderate compared to synthetic films (Zactiti, E. and Kieckbusch, T. J.,2006). Alginate edible-films are appropriated to load additives and anti bacterial compounds. Good results are obtained applying probiotics (Tapia *et al.*, 2007) and oil compunds such as garlic oil (Pranoto *et al.*, 2005) and oregano oil (Rojas-Grau *et al.*, 2007).

2.5.3 Glycerol as an edible film

Glycerol, also known as glycerine or propane-1,2,3-triol, is a chemical which has a multitude of uses in pharmaceutical, cosmetic, and food industries. It can be produced as a by-product from saponification and hydrolysis reactions in oleochemical plants as well as trans esterification reaction in biodiesel plants (Ueoka and Katayama 2001). Glycerol is a simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in all lipids known as triglycerides. It is widely used in the food industry as a sweetener and humectant and in

pharmaceutical formulations. Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature (Christoph *et al.,* 2006).

In food and beverages, glycerol serves as a humectant, solvent, and sweetener, and may help preserve foods. It is also used as filler in commercially prepared low-fat foods (e.g., cookies) and as a thickening agent in liqueurs. Glycerol and water are used to preserve certain types of plant leaves (Stevens A., 2002). Glycerol is used in medical, pharmaceutical and personal care preparations, mainly as a means of improving smoothness, providing lubrication, and as a humectant. It is found in allergen immuno therapies, cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps, and water-based personal lubricants. In solid dosage forms like tablets, glycerol is used as a tablet holding agent. For human consumption, glycerol is classified by the U.S. FDA among the sugar alcohols as a caloric macronutrient (Kong *et al.*, 2016).

2.5.4 Edible film containing products

A novel approach for the development of probiotic baked cereal products was reported by C. Soukoulis *et al.* (2014). Probiotic pan bread constructed by the application of film forming solutions based either on individual hydrogels e.g. 1% w/w sodium alginate (ALG) or binary blends of 0.5% w/w sodium alginate and 2% whey protein concentrate (ALG/WPC) containing *Lactobacillus rhamnosus GG*, followed by an air drying step at 60^0C for 10 min or 180^0C for 2 min were produced. No visual differences between the bread crust surface of control and probiotic bread were observed. The use of film forming solutions based exclusive on sodium alginate improved the viability of *L. rhamnosus GG* under simulated gastro-intestinal conditions, and there was no impact of the bread crust matrix on inactivation rates. Results revealed that an individual 30- 40 g bread slice can deliver approx. 7.57-8.98 and 6.55-6.91 log cfu/portion before and after *In vitro* digestion, meeting the WHO recommended required viable cell counts for probiotic bacteria to be delivered to the human host.

Resistant starch (Hi-maize) at a concentration of 1% was used for the microencapsulation of Lactobacillus acidophilus in alginate beads. Moist and freeze-dried microparticles were obtained. The addition of prebiotics did not increase the size of the moist particles. The Hi-maize provided better protection for the probiotics after exposure to simulated gastrointestinal juice for both the moist and the freeze-dried

microparticles. Regarding the viability of the probiotic culture during storage, both treatments proved to be viable, with suitable values conferring probiotic effects (<6 log CFU g^{-1}), with at least 30 days of stability in the freeze-dried form and 135 days in the moist form, both under storage at room temperature (25 °C) (Mariana *et al.*, 2016).

An innovative approach was performed to prepare novel pullulan /starch blended edible films by direct incorporation of multiple probiotic bacterial strains. Various starches different in origin (potato, tapioca and corn) were blended into the pullulan solutions with different ratios. The physical and mechanical properties of the films were investigated in the presence and absence of probiotic cells. At room temperature (25–27^{0}C), all of the films except starch showed similar cell viabilities, but maximum cell viability was observed in pure pullulan films However, all of the films except starch films maintained cell viabilities up to 20 days, thereafter cell viabilities decreased. For example, the pure pullulan film showed approximately 80% viability after 10 days of storage at 25^{0}C but a substantial decrease to 35% after 20 days. Starch incorporation in the film decreased the cell viability. Among the starches of different origins, potato starch appeared better for cell survival compared to other starches (C. Soukoulis *et al.*, 2016).

C. Soukoulis *et al.* (2014) developed prebiotic edible films as effective vehicles for encapsulating probiotic living cells. Four soluble fibres (inulin, polydextrose, glucose-oligosaccharides and wheat dextrin) were selected as prebiotic co-components of gelatine based matrices plasticised with glycerol and used for the immobilisation of *Lactobacillus rhamnosus GG*. The addition of prebiotics was associated with a more compact and uniform film structure, with no detectable interspaces or micropores; probiotic inclusion did not significantly change the structure of the films. Glucose oligosaccharides and polydextrose significantly enhanced *L. rhamnosus GG* viability during air drying (by 300% and 75%, respectively), whilst a 33% and 80% reduction in viable counts was observed for inulin and wheat dextrin. Results indicated that inulin and wheat dextrin has greater stability (30 & 27 days) during storage than Glucose-oligosaccharides and polydextrose (23 & 28 days).

A study was to obtain functional bread combining the microencapsulation of Lactobacillus acidophilus and starch based coatings. Different probiotic coatings (dispersed or multilayer) were applied onto the surface of partially baked breads. In all

treatments, microencapsulated *L. acidophilus* survived after baking and storage time, although reduction was higher in the sandwich treatment (starch solution/sprayed microcapsules/starch solution (Fortoul *et al.*, 2011).

2.6 Synbiotic

2.6.1 Introduction

Gibson and Roberfroid (1995) proposed the use of probiotics and prebiotics fusion products or "synbiotics" for the intestinal tract microbiota (Panigrahi *et al.*, 2008). A synbiotic has been defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' (Gibson & Roberfroid, 1995).

The main reason for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature. As prebiotics provides a great place for probiotics to thrive, the population of these good bacteria is known to preserve. Studies have shown that by harnessing both the benefits of these prebiotics and probiotics into synergy, the number of good bacteria in the digestive systems increased many folds for the betterment of our health (Bhupinder Singh Sekhon and Saloni Jairath 2010).

Synbiotics work in two ways i) by improving the viability of probiotics and ii) by delivering specific health benefits (Bhupinder Singh Sekhon and Saloni Jairath 2010). The intake of a synbiotic food leads to a modulation of the gut metabolic activities with a maintenance of the gut biostructure. In particular, the significant increase of short chain fatty acids, ketones, carbon disulfide and methyl acetate following the feeding period suggested potential health promoting effects of the synbiotic food (Beatrice *et al.*, 2010).

2.6.2 Products that contain prebiotic, probiotic or both

A novel approach for the development of whey-based probiotic product with *Lactobacillus reuteri* and *Bifidobacterium bifidum* was reported by Adrian *et al.*, (2006). *Lactobacillus reuteri* and *Bifidobacterium bifidum* were inoculated (2.8·108 and 4.7·108

CFU/mL, respectively) into reconstituted whey containing sucrose and pectin in order to prepare a fermented probiotic product. Inoculation levels were: 0.5, 1 or 2 % for *Lactobacillus reuteri* and 0.5 or 1 % for *Bifidobacterium bifidum*. The treatment with the highest bacterial counts and sensory scores was selected and stored at 4 °C for 30 days. Microbial counts, changes in pH values, titratable acidity and both triangle test and sensory attributes were monitored on the stored product. The beverage fermented for approx. 11 h and prepared with 2 % *Lactobacillus reuteri* and 0.5 % *Bifidobacterium bifidum* met the probiotic criterion by maintaining both bacterial populations at counts greater than 10^6 CFU/mL for the whole storage period. Titratable acidity and pH values as well as sensory properties did not change appreciably during the first 14 days of storage. At the end of the storage period (30 days), slight acidification was detected, although the beverage still retained an acceptable flavour.

Ana *et al.*, (2013) investigated the survival ability of *L. casei* in cashew apple juice during refrigerated storage (4°C) for 42 days. Process optimization was done through an experimental design changing initial pH and fermentation temperature. Response surface methodology (RSM) was applied to the response variables (biomass and cell viability). The optimum conditions for probiotic cashew apple juice production were initial pH 6.4, fermentation temperature of 30ºC, inoculums size of 7.48 Log CFU/mL and 16 h of fermentation process. It was observed that the *Lactobacillus casei* grew during the refrigerated storage.

A study was carried out to prepare synbiotic ice cream incorporating *Lactobacillus acidophilus* and inulin and viability of *Lactobacillus acidophilus* was analyzed on storage. Incorporation of inulin in ice cream mix significantly (P<0.01) improved the growth of *Lactobacillus acidophilus*. Freezing of the ice cream mix caused a reduction of 0.61 to 0.77 log counts of *L. acidophilus* count. A significant reduction (P<0.01) in the count of *L. acidophilus* was observed during storage. It is concluded that incorporation of inulin increases count of *L. acidophilus* and the organism could survive at therapeutic minimum probiotic level of 10^6 cells ml for 15 days of storage at -18 to -23°C in ice cream (Pandiyan *et al.*, 2012).

A study was evaluated the effects of adding selected fruits and vegetables local to Mwanza, Tanzania on the sensory qualities of probiotic yogurt supplemented with Moringa oleifera, a local tree with a high micronutrient and protein content. Moringa-

probiotic yogurt was liked significantly less than the control sample for appearance, taste, texture and overall quality. This study showed that by adding banana to Moringa-probiotic yogurt it can be made comparable to probiotic yogurt alone. Moringa-banana probiotic yogurt had the second highest mean score in all categories and had significantly higher ratings than the Moringa-probiotic yogurt for appearance, taste, texture and overall quality (Kuikman & Connor, 2015).

Gustaw *et al.*, (2011) analyzed the influence of inulin and fructooligosaccharides on the growth of lactic acid bacteria The FOS and inulin addition to yoghurt caused an increase in the numbers of all bacteria in comparison to control yoghurt obtained without addition of prebiotics. The viable counts of *Str. thermophilus, Lb. acidophilus* and *Bifidobacterium sp.* when 1% of FOS was added to yoghurt were about 9 log cfu/g, 7.8 log cfu/g and 7.7 log cfu/g, respectively. In the presence of 1% of inulin, *streptococci* and *bifidobacteria* reached the growth at the level 8.8 log cfu/g and 7.5 respectively. Viability of bacteria was sufficient for 14 days and then their numbers decreased but usually not below 10^6 cfu/g. Prebiotics as FOS and inulin added to bio-yoghurt exhibited stimulatory effect on growth *Lb. acidophilus* and *Bifidobacterium sp*. Addition of prebiotics caused an increase in apparent viscosity and hardness (in case of FOS) and decrease in syneresis of obtained bio-yoghurts.

Orange cakes with addition of inulin and oligofructose to justify a prebiotic claim (minimum of 3 g of fructans in a 60 g serving of cake) were investigated by Larissa *et al.*, 2012. The sensory profile of cakes with inulin, with inulin/oligofructose and without prebiotics (standard cake) was evaluated using descriptive quantitative analysis. Preference mapping was assessed using multidimensional scaling on data obtained through an acceptability test with a nine-point hedonic scale. The cakes with prebiotics presented greater crust brownness, hardness and stickiness than the standard cake and lower crumbliness. Principal Component Analysis (69.5 and 10.7% of explanation to the first and second principal components, respectively) showed that crust brownness, dough beigeness, hardness and stickiness contributed to distinguish the cakes with prebiotics from standard cakes. The sensory acceptability was similar for the three cakes and higher when compared to three commercial cakes, but the preference mapping showed that cakes with prebiotics were preferred to commercial cakes.

2.7 *In vitro* digestion models

2.7.1 General introduction

In vitro (*meaning*: in the glass) studies are conducted using components of an organism that have been isolated from their usual biological surroundings, such as microorganisms, cells, or biological molecules. *In vitro* digestion models are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions. In the past few years, there has been an increasing interest in the structural design of food-based delivery systems to encapsulate, protect and release bioactive components believed to benefit human health (McClements *et al.,* 2009). These delivery systems may be designed to release the bioactive components at a specific location in the human gastrointestinal (GI) tract, often in response to an environmental trigger, such as pH, ionic strength, or enzyme activity.

The ideal *In vitro* digestion method would provide accurate results in a short time (Coles *et al.,* 2005) and could thus serve as a tool for rapid screening foods or delivery systems with different compositions and structures. In practice, any *In vitro* method is inevitably going to fail to match the accuracy that can be achieved by actually studying a food in vivo due to the inherent complexity of the process (Coles *et al.,* 2005; Fuller, 1991). Consequently, some compromise is needed between accuracy and ease of utilization of any *In vitro* digestion model. During the past few years, food and animal scientists have utilized a number of *In vitro* digestion models to test the structural and chemical changes that occur in different foods under simulated GI conditions, although none of these methods has yet been widely accepted.

The *In vitro* digestion models may differ from one another in their operation. According to Hur *et al.,* 2011 those include:

(1) The number and type of steps included in the digestion sequence, e.g., mouth, stomach, small intestine, large intestine.
(2) The composition of the digestive fluids used in each step, e.g., enzymes, salts, buffers, biological polymers and surface-active components.

(3) The mechanical stresses and fluid flows utilized in each step in the digestion sequence, e.g., magnitude and direction of applied stresses, flow geometries and flow profiles.

In addition, there are considerable differences in the type of experimental parameters measured in the various digestion models. These include chemical changes (such as hydrolysis of lipids, proteins and/or polysaccharides), location changes (such as release of encapsulated components, competitive adsorption processes, multilayer formation) and structural changes (such as breakdown of specific structures, aggregation, droplet coalescence, or droplet disruption).

The most frequently utilized enzymes and other biological molecules used within *In vitro* digestion models are pepsin, pancreatin, trypsin, chymotrypsin, peptidase, a-amylase, lipase, bile salt and mucin.

2.7.2 *In vitro* digestion and sample conditions

The characteristics of foods, enzyme type, and enzyme concentrations are key factors that control the digestion of foods during *In vitro* digestion. Abdel-Aal (2008) reported that the differences in digestibility reflect influences of proteolytic enzymes, digestion conditions, as well as the status of protein sources (raw versus processed). Increase in dietary protein induces an increased secretion of pancreatic proteolytic enzymes, while an increase in starch or lipid intake induces increased secretions of amylase and lipase, respectively (Boisen & Eggum, 1991). Thus, *In vitro* digestion characteristics such as digestion time, enzyme contents, or enzyme composition must be adjusted according to sample characteristics. For instance, if the concentration of the target substance (protein, lipid, or carbohydrate) is increased, then the concentration of enzymes or the digestion time must be increased even if the rests of the *In vitro* digestion procedure is kept the same. However, Green *et al.*, (2007) reported that the addition of digestive enzymes did not significantly alter the amount of catechin recovered from green tea after passing through an *In vitro* digestion model. They found that the amount of catechin recovered was similar using an *In vitro* digestion model containing digestive enzymes, as had been reported using an approach that used no enzymes (Record & Lane, 2001). This may be because humans (monogastric stomach) cannot digest plant-based foods well, and so the presence or absence of enzymes had little impact on the release of catechin.

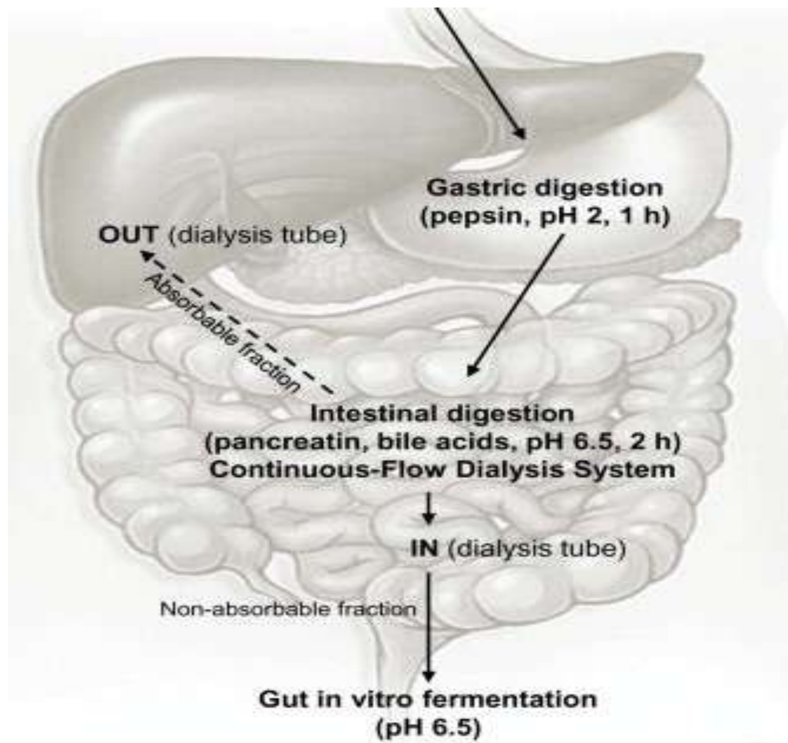


Fig. 2.1: *In vitro* digestion method for food matrix

CHAPTER III

MATERIALS AND METHODS

The study was conducted in the Food Processing and Engineering laboratories- 1and 2 under Faculty of Engineering in collaboration with Microbiology laboratory under the Faculty of Veterinary and Animal Science of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from January 2017 to July 2017.

3.1 Materials

3.1.1 Sources of raw materials

Garlic was collected from local market at Basherhat for extracting prebiotic. All ingredients (White flour, sugar, powder milk, fresh whole eggs, baking powder and vanalia) for preparing cakes were collected from local market at Basherhat, HSTU campus.

3.1.2 Chemicals

The chemical and reagents used during the research work were obtained from the companies listed below:

- Ethanol (Merck, Germany)
- Calcium carbonate(Merck, India)
- Lactobacilli MRS Agar(Merck, Germany)
- Lactobacilli MRS broth(Merck, Germany)
- Sodium alginate(Merck, Germany)
- Glycerol(Merck, Germany)
- Pepsin (Loba Scientific, India)
- NaCl (Merck, India)
- KCl (Merck, India)
- NaOH (Merck, India)
- Pancreatin (Merck, Germany)
- Bile salts (Loba Scientific, India)

3.1.3 Apparatus

- UV-VIS spectrophotometer (PG instruments T60, UK)
- Electrical balance, (Mettler, Toledo AB 104).
- Shaking incubator (Vision Scientific, model: VS-8480SN, South Korea)
- Muffle furnace
- Electrical balance, (Mettler, Toledo AB 104).
- Oven, IH-150, (Gallenkamp, England)
- Digital pH meter (HI-98107)
- Magnetic stirrer
- Grinding Machine
- Centrifuge
- Homogenizer (VELP Scientifica, Italy)
- Water bath
- Filter cloth
- Filter paper (Whatman No. 1)
- Petri dish
- Test tube
- Crucible

3.2 Detailed methodology of synbiotic cake

3.2.1 Extraction of prebiotic

Inulin extraction:

Firstly, samples were boiled for 5 min to eliminate enzymes. Then they were stoved at 60°C for 7 h and smashed witha grinder. The sample power was boiled in deionized water at 90°Cfor 40 min twice and filtered. $Ca(OH)_2$was added to the filtrate until pH reached 11 to remove the protein, and $H_3PO_4$was added until pH was 8 to remove the redundant $Ca(OH)_2$. Then 30% H_2O_2 (v/v 3%) were used to bleach the solution. Finally, the inulin powder was collected by precipitation with excess ethanol and oven dried at 60°C.

Fig. 3.1: Sorted garlic

Fig. 3.2: Filtrate after filtering

Fig. 3.3: Extracted inulin

3.2.2 Culture of probiotic

Procedure

To obtain direct counts of lactobacilli, 15 - 20 mL sterile, molten (45 - 50°C) Lactobacilli MRS (de Man - Rogosa – Sharpe) agar poured into sterile petri dishes containing 1 mL volumes of diluted test sample. Agar was distributed throughout medium by rotating the plate in one direction, then in the reverse direction. Then allowed medium to solidify on a flat surface for 5 - 10 minutes. Agar plates were incubated at 35°C for 2 days in an anaerobic atmosphere. Probiotic stock cultures were prepared by mixing cells with MRS broth (1:1 v/v). For regeneration, 0.1 mL of sample were aseptically transferred to 10 mL of MRS broth and incubated at 37° C.

Fig. 3.4: Fresh milk

Fig. 3.5: Molten agar pouring

Fig. 3.6: Lactobacillus culture

3.2.3 Preparation of probiotic cells

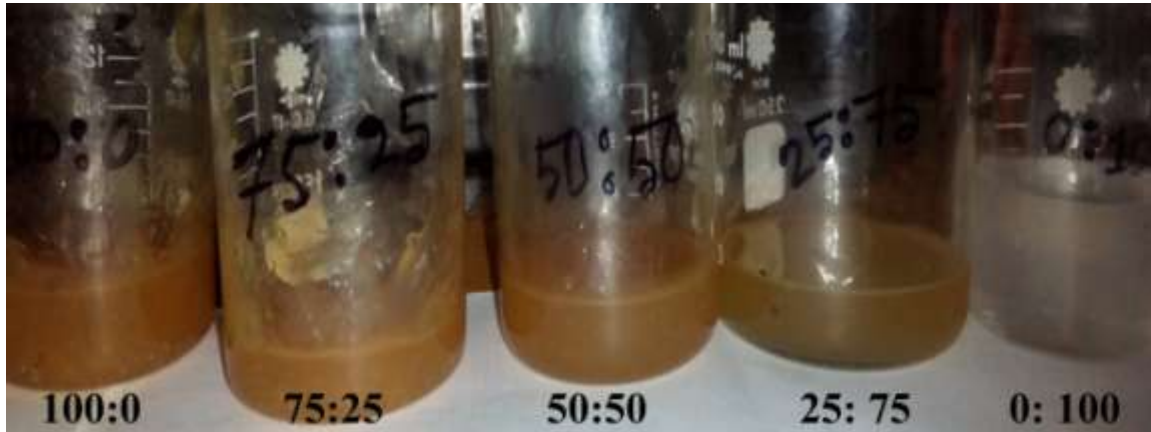
Preparation of probiotic cell was done according to the procedure described by Kanman & Lim (2013). Five beads of the cultures were placed in 25 mL of MRS broth and incubated at 37^{0}C overnight. From this, 10 mL of culture were aseptically transferred to

500 mL of MRS broth in a 25 ml conical flask and kept at 37^0C with shaking. After 16 h of incubation, the whole probiotics cell biomass was harvested by centrifugation at 3000 rpm for 15 min . After centrifugation, the supernatant was discarded and the harvested cells in the form of pellets were washed twice using phosphate buffer saline pH 7.0. and re-suspended in 20 mL of deionised water for film application. One mL of each probiotic cell suspension was directly incorporated in film forming solution into these mixture solutions.

Fig. 3.7: Probiotic cell

3.2.4 Preparation of the film forming solutions

Film forming solution was prepared according to Kanmani & Lim (2013). Solutions sodium alginate (20 g) and glycerol (5 g) were prepared separately by dissolving each in 100 mL of deionised water while stirring was done with a magnetic stirrer at room temperature. Subsequently, sodium alginate and glycerol solutions were mixed in the ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (v/v) at 95˚ C and 160 rpm. Following this, all the blended solutions were autoclaved at 121^0 C for 15 min and allowed to cool at room temperature. The mixtures were then centrifuged at 3000 rpm for 2 min, in order to remove air bubbles from the solutions. Then, the film forming solutions was cool down to 25^0C. One mL of each probiotic cell suspension was directly incorporated into these mixture solutions.



Sodium alginate: Glycerol

Fig. 3.8: Film forming solution

3.2.5 Preparation of symbiotic cakes

White wheat flour, inulin,sugar, powder milk, fresh whole eggs, inulin, baking powder and vanalia were used to formulate cake. All ingredients were mixed for 10 min using a mixer. All ingredients of cake were placed into metallic pans, and were baked in an electric oven for 30 minutes at 180°C. The cakes were cooled after baking. A small amount (0.5 ml) of probiotic edible film forming solution were applied and uniformly distributed by brushing on the crust of the cake loaves. The synbiotic cake was then rapidly dried at 60^0 C for 10 min in an oven. After the completion of the drying step, the cake samples were left to cool to room temperature (25^0 C) and were packed in high density polythene (HDPE) in tight condition.

Fig. 3.9: Batter with inulin

Fig. 3.10: Batter in a pan

Fig. 3.11: Prepared cake

Fig:3.12 Application of film forming solution

Fig. 3.13: Synbiotic cake

3.2.6. Research design :

Two factor completely randomized design (CRD) was employed for conducting this research.

3.1. Table: Research design of formulated cake

Factor	Level	Responses
Inulin (T)	1% inulin	Moisture content, pH, fiber content, firmness, color, Enumeration of bacteria
	2% inulin	
	3% inulin	
Film forming solution (S) (sodium alginate: glycerol)	100:0	
	75:25	
	50:50	
	25:75	
	0:100	

Table-3.3: Formulations of synbiotic cakes :

The amount of Wheat flour (100 gm), sugar(100 gm), Egg(100 gm), powder milk(5 gm), baking powder(5gm), Vanilla essence (5 gm), Soybean oil(100 gm) were kept in constant.

Sample	INGREDIENTS	
	Inulin (g)	Film forming solution (Sodium alginate: Glycerol)
Control	-	-
T_1S_1	4	100:0
T_1S_2	4	75:25
T_1S_3	4	50:50
T_1S_4	4	25:75
T_1S_5	4	0:100
T_2S_1	8	100:0
T_2S_2	8	75:25
T_2S_3	8	50:50
T_2S_4	8	25:75
T_2S_5	8	0:100
T_3S_1	12	100:0
T_3S_2	12	75:25
T_3S_3	12	50:50
T_3S_4	12	25:75
T_3S_5	12	0:100

3.2.6 Characterization of inulin

3.2.6.1 Functional properties

3.2.6.1.1 Determination of swelling capacity (mL of water/g of sample)

The swelling capacity of cake was determined by the method of Okaka and Potter (1977) with some modifications. The sample filled up to 10 ml mark in a 100 ml graduated cylinder. Then, water was added to adjusted total volume to 50 ml of cylinder. Then top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. After 2 min later the suspension was inverted again and allowed to stand for further 30 min. The volume occupied by the sample was taken after 30 min.

3.2.6.1.2 Determination of Water absorption index (g of water/g of sample)

The water absorption index (WAI) content was measured by the method of Sosulski *et al.* (1976) with some modification. Inulin (0.4g) was suspended with 10 ml of water into a 15 ml centrifuge tube. Then the mixture was centrifuged for 20 min at 3500 rpm and the supernatant was poured carefully into a petridish. The residue was weighed. Water absorption index was determined by following formula:

$$\text{WAI (g/g)} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of tube with residue

W_2 = weight of the tube

W = weight of sample

3.2.6.1.3 Solubility (g/L)

The solubility of the inulin was determined according to the Cano-Chauca *et al.* (2005) with some modification. One gram inulin and 100 ml of distilled water was homogenized by a magnetic stirre for 5 min at high speed. Then the solution was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. An aliquot of 25 ml of the supernatant was transferred to pre-weighed petridishes and oven-dried at 105°C overnight. The solubility was calculated by weight difference and expressed as percentage.

3.2.6.2 Chemical properties of inulin

3.2.6.2.1 Determination of moisture content (%)

AOAC method (2000) was used to determine the moisture content of inulin. Three gram inulin was taken in a clean, dry and pre-weighted petridish. Then the sample was transferred to oven and dried at 105ºC for 24 hours. After that it was cooled at desiccator and weighed. Moisture content was calculated by following formula:

$$\text{\% Moisture} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of sample with crucible

W_2= weight of dried sample with crucible

W = weight of sample

3.2.6.2.2 Ash content

The ash content was determined by the method of AOAC (2000). Muffle furnace was used to determination of the ash content. At first 5g sample was weighed and transferred into a clean, dry and pre-weighted crucible. Then the crucible with sample placed in a muffle furnace and dried the sample at 550°C for 5-6 hrs. When the time was accomplished, the sample with crucible cooled in desiccator for 15 minutes and again weighted. To ensure the completion of ashing, the crucible was again transferred in muffle furnace for half an hour and then cooled in desiccator and weighed again. This process was repeated until a constant weight was obtained and the ash became almost white in color. The ash content was calculated by the following formula:

$$\% \text{ Ash} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of ash with crucible

W_2 = weight of empty crucible

W = weight of sample

3.2.6.2.3 Determination of pH

pH value of the inulin was measured according to the method AOAC (2000) with few modifications. Digital pH meter was used to determine the pH value of the sample by performing two point calibration (with buffer 7.0 and buffer 4.0) before measuring the sample pH. After calibration electrode assembled pH meter was dipped into the inulin solution; the pH was then readout.

3.2.6.2.4 Total soluble solids (mg/ml)

Total soluble solids of inulin were determined according to Dubois *et al.*, (1956). At first 500 mg inulin was taken. Then added 10 ml ethanol and homogenized the sample. After homogenizing, the sample was centrifuged at 2000 rpm for 20 min. The supernatant of 2 ml was mixed with 1 ml of phenol solution. Subsequently 5ml of H_2SO_4 was mixed with the sample. The test tubes kept stand for 10 mins. After that the test tubes were vortexed for 30sec.Then test tubes were kept at room temperature for 20 min for color

development. Absorbance were taken at 490 nm. Standard curve for total soluble sugar determination was constructed using glucose solutions whose concentrations ranged between 0 to 0.25 mg/ml.

3.2.6.2.5 Reducing sugar (mg/ml)

Reducing sugar was estimated by 3, 5- dinitrosalicylic acid (DNS) method (Miller, 1959). Inulin were mixed with 10 ml ethanol and homogenized. After that the solution was centrifuged at 2000 rpm for 20 min. Then 0.5 ml of supernatant was mixed with 0.5ml of DNS solution .The mixture was boiled for 10 min. The test tube was cooled by immersing the sample into cold water. 5 ml of water was added and mixed well. Afterward, the absorbance was taken at 540nm in a UV spectrophotometer. The standard curve using for determination of the reducing sugar content was developed by using glucose solutions whose concentrations ranged between 0 to 1.2 mg/ml.

3.2.6.2.6 Non reducing sugar (mg/ml)

Non reducing sugar was estimated by subtracting the reducing sugar content from the total soluble sugar content by using the following formula:

Non reducing sugar = Total soluble sugar – Reducing sugar

3.2.6.2.7 Color

Color of the product was evaluated by a colour measurement spectrophotometer (Minolta Camera, Tokyo, Japan) set for Hunter L*(lightness), a* (redness) and b* (yellowness) values. L* is measured on scale of 0=black to 100=white, a* measures red to green with + a being red, and -a being green, and b* measures yellow to blue with +b being yellow and -b being blue. The results of the Hunter L*, a* and b* values were averaged from 2 replications.

3.2.7 Quality analysis of prepared cakes

3.2.7. 1 Physical properties

3.2.7.1.1 Firmness of cake

Firmness was measured with penetrometer (Gy-4, china). An 8 mm diameter cylindrical probe was penetrated into cake surface. After that automatic data was shown on penetrometer screen. Data was expressed as N.

3.2.7.1.2 Determination of color

Color of the product was evaluated by a colour measurement spectrophotometer (Minolta Camera, Tokyo, Japan) set for Hunter L*(lightness), a* (redness) and b* (yellowness) values. L* is measured on scale of 0=black to 100=white, a* measures red to green with +a being red, and -a being green, and b* measures yellow to blue with +b being yellow and -b being blue. The results of the Hunter L*, a* and b* values were averaged from 2 replications.

3.2.7. 2 Chemical properties

3.2.7. 2.1 Determination of moisture content (%)

AOAC method (2000) was used to determine the moisture content of cake. Moisture Content was calculated by following formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of sample with crucible

W_2= weight of dried sample with crucible

W = weight of sample

3.2.7. 2.2 Determination of pH

pH value of the prepared cakes were measured according to the method AOAC (2000) with few modifications.

3.2.7. 2.3 Determination of fiber content (%)

The cake samples were taken for crude fiber analysis by adopting the procedure mentioned in AOAC (2000) Method No. 32- 10. 5g sample was used to determine crude fiber of cake. Samples were boiled for 30 minutes in the presence of 1.25% H_2SO_4 and then filtered and washed. Then these samples were again boiled in 1.25% NaOH for

30minutes and then filtered and washed. The resultant residue was dried at 110ºC for 2 hours and weighed. The dried residue was ignited at 550 ± 15ºC, cooled and reweighed. The crude fiber was calculated according to following expression:

$$\% \text{ Fiber} = \frac{\text{Loss in weight on ignition}}{\text{Weight of sample}} \times 100$$

3.2.7.3 Microbiological analysis of symbiotic cake

Enumeration of the bacteria (log CFU/g):

One gram of *Lactobacillus spp.* containing cake crust samples were transferred to 9 mL of sterile PBS and left to hydrate and dissolve under constant agitation in an orbital incubator at 37ºC for 1 hour. The resulting solutions were subjected to serial dilutions using phosphate buffer saline. Each dilution was pour plated on a MRS agar and the plates were stored at 37ºC for 72 hour under anaerobic conditions to allow colonies to grow. Enumeration of the bacteria was performed in triplicate and the total counts of the viable bacteria were expressed as log colony forming units per gram (log CFU/g).

$$\text{Total bacterial count} = \frac{\text{Number of colonies on each plate} \times \text{Reciprocal of dilution}}{\text{Aliquot taken}}$$

3.2.7.4 *In vitro* digestion (log CFU/g)

Probiotic cake crust systems and free bacteria with or without cake crust were compared for their ability to survive *In vitro* digestion simulating the human gastric and intestinal environments. The method was based on a previously published procedure (Yonekura & Nagao, 2009), with modifications. 2mg of cake crust was mixed in a saline solution containing 140 mM NaCl, 5 mM KCl and 150 mM BHT at a ratio of 1:4 v/v (sample/saline) to obtain a final volume of 14 mL. This was followed by agitation at 21 ^{o}C for 10 min. Afterward, the mixture was acidified to pH 2.0 with 0.1 M/1 M HCl and was added to a 0.250 mL solution containing pepsin (0.2 g in 5 mL 0.1 M HCl), and the samples were incubated at 37°C with stirring for 1 h.

After step-wise gastric digestion, the intestinal digestion of the sample was commenced by raising the pH to 6.9 by the addition of 1 M $NaHCO_3$/0.1 M NaHCO3. Then, 1.25 mL bile and pancreatic solution (containing 0.225 g bile extract and 0.0375 g pancreatin in a volume of 18.75 mL 0.1 M$NaHCO_3$) was added followed by incubation with shaking at

$37^{o}C$ for 2 h. The final volume of the digested sample was adjusted to 14 mL by the addition of brine. From this 1ml aliquots of the digesta were diluted in 9 mL of PBS and plated on MRS agar for enumeration of viable bacteria, as described above.

3.3 Statistical Analysis

Experiments were performed in triplicate .The statistical analysis of the data was performed by analysis of variance (ANOVA) with a significance level of 5% using SPSS, version 22.0. Two-way ANOVA followed by Duncan's post hoc means comparison ($p < 0.05$) test was performed to evaluate the interaction effects of edible film containing probiotic and inulin on the micro- biological data.

3.4 Sensory evaluation of synbiotic cake

Sensory evaluation of the formulated cakes was carried out at the Faculty of Engineering, Hajee Mohammad Danesh Science and Technology University by 15 consumers (untrained panelists) including teachers, students and staff, selected based on interest. The panelist was requested to assign score for characteristics texture, crust brownness, crust uniformity, taste and flavor.

The scale was arranged such

9 = Like extremely;

8 = Like very much;

7 = Like moderately;

6 = Like slightly;

5 = Neither like nor dislike;

4 = Dislike slightly;

3 = Dislike moderately;

2 = Dislike very much;

1 = Dislike extremely

3.5: Storage study:

Storage stability was evaluated at 0, 4 and 8 days. All prepared symbiotic cake was kept at room temperature and physicochemical, microbial, and sensory evaluation were conducted after 8 days.

CHAPTER IV

RESULTS AND DISCUSSION

This research was conducted in the Food Process and Engineering laboratories 1and 2 under the faculty of Engineering with the collaboration of Microbiology laboratory. The aim of this effort was to find out the physicochemical and microbiological characteristics of developed synbiotic cake compared to plain cake. The results obtained from the study are presented and discussed in this chapter under the following headings.

4.1 Characterization of extracted inulin

Physico-chemical and functional properties of extracted inulin are illustrated in Table-4.1, 4.2 and 4.3.

4.1.1 Physico-chemical properties

The physico-chemical composition of inulin is presented in Table (1 and 2) such as moisture, ash, pH, total sugar, reducing sugars, non reducing sugar and color.

4.1.1.1 Moisture content (%)

Table-4.1 presents the mean value of moisture content (5.82 ± 0.04%) for inulin. Azza *et al.* (2011) studied on moisture content (5.89 ± 0.01) of inulin produced from Jerusalem artichoke who demonstrated higher result as compared to this findings. The value (5.82%) was higher than the moisture (3.5%) of inulin extracted from Jerusalem artichoke tubers reported by Niness (1999).

4.1.1.2 Ash content (%)

The mean values and standard error of ash content of extracted inulin was 0.58 ± .05% (Table 4.1). Our finding was lower than inulin (2.89%) extracted from *Agave americana* leaves (Mohamed *et al*, 2014). Similar value (0.485%) was found by Azza *et al.* (2011) who reachersed on inulin from Jerusalem artichoke. Niness (1999) supported the present study who revealed the inulin properties from Jerusalem artichoke tubers.

4.1.1.3 pH

The pH of inulin is displayed in table-4.2. This outcome (5.52 ± .01) exhibited quite similar than the result of pH (5 - 7%) found from standard chicory inulin (Molina *et al.*, 2005). Mohamed *et al.* (2014) revealed the similar result for pH (5.53 ± 0.55).

4.1.1.4 Total sugar (mg/ml)

Inulin showed to have 0.07 ± 0.01 mg/ml of total sugar (Table-4.1). This property is in accordance with Toneli *et al.* (2008) who clarified related result for inulin extracting from chicory root. Franck (2002) and Elok and Wilda (2013) reported the percentage of total sugar 8 and 5.6 mg/ml respectively for chicory root and dahlia.

4.1.1.5 Reducing and non-reducing sugar

The data of reducing and non-reducing sugar are summarized in Table-4.1.The value 0.04 ± 0.004 mg/ml stood for reducing sugar and 0.03 ± 0.01 for non-reducing sugar. Similar trends were observed by Azza *et al.* (2011) who described inulin having 0.052 mg/ml and 0.048 mg/ml of reducing and non-reducing sugar.

4.1.1.6 Color

Color value of extracted inulin was 68.95 ± 0.69, 0.13 ± 0.01 and 7.50 ± 0.02 respectively for L*, a* and b*(table-4.2). a* value was related to the previous studies Azza *et al.* (2011) who found 0.12 for Jerusalem artichoke inulin . On the contrary, our results (L* and b*) were higher than that of inulin extracted from Jerusalem artichoke reported by Azza *et al.* (2011).

4.1.2 Functional properties of inulin

Table-4.3 illustrates the functional properties of inulin obtained from garlic.

4.1.2.1 Solubility (g/L)

The solubility (71.64± 1.00 g/L) of inulin is presented in table-4.3. Similar behavior (73.47 ± 1.4g/L) was reported by Mohamed *et al.* (2014) who extracted inulin from *Agave americana* leaves. In contrast, this value was lower (120 g/L) than chicory inulin which was summarized by Molina *et al.* (2005).

4.1.2.2 Water absorption capacity (g of water/g of sample)

Water absorption capacity is a vital functional property of inulin. It is clearly demonstrated from table-4.3 that our analytical result was 2.16 ± 0.02 g of water/g of sample. The reported value is consistent with the findings (2.42 ± 0.18 g/L) of Mohamed *et al.* (2014) for Agava inulin. On the contrary, Azza *et al.* (2011) assigned lower records (0.52 g/L) for inulin extracted from Jerusalem artichoke. The high WAC inulin suggest that it can be used as a functional ingredient to improve the sensory properties of the formulated product, to reduce syneresis, modify texture, viscosity, and reduce calories of foods (Mohamed *et al.*, 2014).

4.1.2.3 Swelling capacity (mL of water/g of sample)

Swelling capacity determines the extent to which sample increases in volume in relation to its initial volume when soaked in water (Baljeet *et al.*, 2014). The swelling property of inulin (1.57 ± 0.01 mL of water/g of sample) is given in table-4.3. Similar observation (1.99 ± 0.13 mL of water/g of sample) was presented by Mohamed *et al.* (2014) who found this conclusion for Agava inulin.

Table-4.1: Chemical properties of inulin

Inulin	Moisture (%)	Ash (%)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Non reducing sugar (mg/ml)
	5.82 ± 0.04	0.58 ± 0.05	0.07 ± 0.01	0.04 ± .004	0.03 ± 0.01

Values are expressed as mean ± SE.

Table-4.2: Physical properties of inulin

Inulin	pH	Color		
		L^*	a^*	b^*
	5.52 ± 0.01	68.95 ± 0.69	0.13 ± 0.01	7.50 ± 0.02

Values are expressed as mean ± SE.

Table-4.3: Functional properties

Inulin	Solubility g/L	Water absorption capacity (g of water/g of sample)	Swelling capacity (mL of water/g of sample)
	71.64 ± 1.00	2.16 ± 0.02	1.57 ± 0.01

. Values are expressed as mean ± SE

4.2 Physico- chemical properties of synbiotic cake

The chemical composition of formulated synbiotic cake is given below. Data regarding chemical characteristics of cake have been presented in Table-4.4, 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10.

4.2.1 Moisture content (%)

Table-4.4: Effects of inulin and film forming solution ratio on moisture content of synbiotic cake (%) at different storage duration

Sample	Storage		
	0 Day (%)	4 Days (%)	8 Days (%)
Control	$^{A}10.44 \pm 0.12^{i}$	$^{A}10.56 \pm 0.14^{j}$	$^{A}10.64 \pm 0.15^{l}$
T_1S_1	$^{A}11.56 \pm 0.36^{h}$	$^{A}11.63 \pm 0.04^{i}$	$^{A}11.87 \pm 0.02^{k}$
T_1S_2	$^{A}11.67 \pm 0.11^{gh}$	$^{A}12.0 \pm 0.03^{i}$	$^{A}12.36 \pm 0.12^{j}$
T_1S_3	$^{A}12.84 \pm 0.14^{f}$	$^{A}12.9 \pm 0.16^{h}$	$^{A}13.29 \pm 0.39^{h}$
T_1S_4	$^{A}14.43 \pm 0.36d^{e}$	$^{A}14.64 \pm 0.38^{f}$	$^{A}14.90 \pm 0.36^{g}$
T_1S_5	$^{A}16.54 \pm 0.23^{c}$	$^{A}16.75 \pm 0.15^{cd}$	$^{A}16.93 \pm 0.17^{c}$
T_2S_1	$^{A}12.47 \pm 0.20^{fg}$	$^{A}12.54 \pm 0.10^{i}$	$^{A}12.67 \pm 0.10^{i}$
T_2S_2	$^{A}13.80 \pm 0.09^{e}$	$^{A}13.87 \pm 0.02^{g}$	$^{A}13.95 \pm 0.12^{h}$
T_2S_3	$^{A}14.47 \pm 0.18^{d}$	$^{A}14.7 \pm 0.04^{ef}$	$^{A}15.14 \pm 0.25^{e}$
T_2S_4	$^{A}16.83 \pm 0.18^{c}$	$^{A}17.0 \pm 0.28^{cd}$	$^{A}17.16 \pm 0.28^{cd}$
T_2S_5	$^{A}17.88 \pm 0.09^{b}$	$^{A}17.9 \pm 0.11^{b}$	$^{A}18.00 \pm 0.08^{b}$
T_3S_1	$^{A}14.39 \pm 0.35^{d}$	$^{A}14.7 \pm 0.13^{ef}$	$^{A}14.88 \pm 0.09^{f}$
T_3S_2	$^{A}14.97 \pm 0.16^{d}$	$^{A}15.2 \pm 0.03^{e}$	$^{A}15.25 \pm 0.20^{e}$
T_3S_3	$^{A}16.43 \pm 0.10^{c}$	$^{A}16.60 \pm 0.08^{d}$	$^{A}16.68 \pm 0.08^{cd}$
T_3S_4	$^{A}16.99 \pm 0.12^{c}$	$^{A}17.20 \pm 0.03^{c}$	$^{A}17.26 \pm 0.25^{c}$
T_3S_5	$^{A}18.68 \pm 0.46^{a}$	$^{A}18.9 \pm 0.33^{a}$	$^{A}19.88 \pm 0.04^{a}$
LSD	0.244	0.553	0.207

Here,

Control = cake without prebiotic and probiotic	**T_1S_1**= cake containing 1% inulin + (100% sodium alginate+ 0% glycerol)
T_1S_2 = cake containing 1% inulin + (75% sodium alginate+ 25% glycerol)	**T_1S_3** = cake containing 1% inulin + (50% sodium alginate+ 50% glycerol)
T_1S_4 = cake containing 1% inulin + (25% sodium alginate+ 75% glycerol)	**T_1S_5** = cake containing 1% inulin + (0% sodium alginate+ 100% glycerol)
T_2S_1 = cake containing 2% inulin + (100%	**T_2S_2**= cake containing 2% inulin + (75%

sodium alginate + 0% glycerol)	sodium alginate+ 25% glycerol)
T_2S_3= cake containing 2% inulin + (50% sodium alginate+ 50% glycerol)	**T_2S_4**= cake containing 2% inulin + (25% sodium alginate+ 75% glycerol)
T_2S_5= cake containing 2% inulin + (0% sodium alginate+ 100% glycerol)	**T_3S_1**= cake containing 3% inulin + (100% sodium alginate+ 0% glycerol)
T_3S_2= cake containing 3% inulin + (75% sodium alginate+ 25% glycerol)	**T_3S_3**= cake containing 3% inulin + (50% sodium alginate+ 50% glycerol)
T_3S_4= cake containing 3% inulin + (25% sodium alginate+ 75% glycerol)	**T_3S_5**= cake containing 3% inulin + (0% sodium alginate+ 100% glycerol)

Values are expressed as mean ± SE. A = (Uppercase) Mean followed by different superscript alphabets in each row is significantly different during storage time ($p < 0.05$).
a–l =(lowercase) Means followed by different superscript alphabets in each column are significantly different among samples ($p < 0.05$).
**Means values in the same column or row showing the same superscript are not significantly different ($P>0.05$).

Table 4 demonstrates a simple comparison on moisture content of the synbiotic cake with control sample. A significant difference ($p < 0.05$) was noted among the samples. The highest moisture value (18.68± 0.46%) was observed for T_3S_5 when lowest value was 10.44 ± 0.12 % for control sample. The result was revealed that our result (moisture content) was lower as compared to cake containing watermelon rind (25.24 to 27.04 %) mentioned by Hanan and Ahmed (2013). This result was in line with the opinion of Aline *et al.* (2013) who found almost similar moisture content (13.64 to 16.00%) for carrot leaf sponge cake. The result revealed that increasing trend of moisture content was observed with the increasing percentage of glycerol because glycerol usually raises the moisture content of product (Warburton and Pixton, 1975). Additionally, increasing the inulin percentage also elevated the moisture content between the samples. Products with high fiber content have high absorbency of water (Torbica *et al.*, 2010).

Throughout the accelerated storage (0 to 8 days), there were no significant differences ($p < 0.05$) between synbiotic samples as well as control sample in terms of moisture content. Previous study by Offia. and Edide (2013) showed increased moisture content (29.22 to 30.97%) of pineapple cake during storage.

4.2.2 pH value

Regarding pH value, formulated cakes showed significant ($P <0.05$) difference (table -5). Control sample differed from others by presenting highest pH (10.05 ±0.003), while T_3S_5 sample (9.79 ±0.008) had the lowest one. With the increasing of percentage of

sodium alginate in film forming solution, pH of cake was decreased. Moreover, with the increasing of inulin percentage pH value of cake was also decreased as inulin is slightly acidic in nature. The results are in comparable with the findings of Jun Ho Lee (2015) who observed lower pH (4.05 to 8.23) for sponge cakes with *Rubus coreanus* powder.

The storage period showed significant ($P < 0.05$) effect on pH of cakes. A reduction in the pH was reported with the passage of storage time. pH of formulated synbiotic samples were decreased due to addition *Lactobacillus spp.* with inulin. *Lactobacillus spp.* utilize carbohydrate especially sugars (e.g., glucose) to form lactic acid. 1 mole of glucose should produce 2 moles of lactic acid, the actual yield is closer to 1.8 moles of lactic acid (Gottshalk, 1986). Our findings are in accordance with Analie and Viljoen (2012), who reported a decrease of pH for yoghurt during storage. Increased production of lactic acid by *Lactobacillus spp.* decreases the pH of the product is in agreement with the results of Salwa *et al.,* (2000).

Table-4.5: pH content of prepared synbiotic cake

Sample	Storage Periods		
	0 Day	4 Days	8 Days
Control	$^{A}10.05 \pm 0.003^{a}$	$^{B}10.04 \pm 0.034^{a}$	$^{B}9.99 \pm 0.032^{a}$
T_1S_1	$^{A}10.02 \pm 0.007^{b}$	$^{B}9.98 \pm 0.021^{b}$	$^{C}8.97 \pm 0.007^{b}$
T_1S_2	$^{A}10.01 \pm 0.001^{c}$	$^{B}9.97 \pm 0.072^{c}$	$^{C}8.88 \pm 0.007^{c}$
T_1S_3	$^{A}9.99 \pm 0.003^{d}$	$^{B}9.96 \pm 0.015^{d}$	$^{B}8.86 \pm 0.006^{d}$
T_1S_4	$^{A}9.97 \pm 0.007^{e}$	$^{B}9.94 \pm 0.021^{e}$	$^{C}8.84 \pm 0.012^{e}$
T_1S_5	$^{A}9.96 \pm 0.012^{f}$	$^{B}9.92 \pm 0.013^{f}$	$^{C}8.83 \pm 0.003^{e}$
T_2S_1	$^{A}9.92 \pm 0.003^{g}$	$^{B}9.87 \pm 0.033^{g}$	$^{C}8.81 \pm 0.023^{f}$
T_2S_2	$^{A}9.91 \pm 0.003^{h}$	$^{B}9.86 \pm 0.061^{h}$	$^{C}8.80 \pm 0.008^{g}$
T_2S_3	$^{A}9.89 \pm 0.004^{i}$	$^{B}9.84 \pm 0.025^{i}$	$^{C}8.78 \pm 0.003^{h}$
T_2S_4	$^{A}9.88 \pm 0.023^{j}$	$^{B}9.83 \pm 0.013^{j}$	$^{C}8.75 \pm 0.010^{i}$
T_2S_5	$^{A}9.86 \pm 0.003^{k}$	$^{B}9.81 \pm 0.057^{k}$	$^{C}8.73 \pm 0.003^{j}$
T_3S_1	$^{A}9.85 \pm 0.001^{l}$	$^{B}9.78 \pm 0.01^{l}$	$^{C}7.73 \pm 0.003^{k}$
T_3S_2	$^{A}9.84 \pm 0.003^{l}$	$^{B}9.77 \pm 0.01^{m}$	$^{C}7.72 \pm 0.050^{l}$
T_3S_3	$^{A}9.82 \pm 0.001^{m}$	$^{B}9.76 \pm 0.03^{n}$	$^{C}7.70 \pm 0.032^{m}$
T_3S_4	$^{A}9.81 \pm 0.003^{n}$	$B9.74 \pm 0.01^{o}$	$^{C}7.68 \pm 0.007^{n}$
T_3S_5	$^{A}9.79 \pm 0.0080^{n}$	$B9.73 \pm 0.01^{p}$	$^{C}7.67 \pm 0.007^{n}$
LSD	0.005	0.008	0.003

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4. Values are expressed as mean ± SE.

A–C =Means followed by different superscript alphabets in each row are significantly ($p < 0.05$) different during storage time.

a–n =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples.

**Means values in the same column or row showing the same superscript small letter is not significantly different ($p > 0.05$).

4.2.3 Fiber content (%)

Table-4.6: Fiber content (%) of formulated cakes

Sample	Storage Periods		
	0 Day	4 Days	8 Days
Control	$^{A}2.65 \pm 0.019^{d}$	$^{A}2.64 \pm 0.023^{d}$	$^{A}2.61 \pm .018^{d}$
T_1S_1	$^{A}2.87 \pm 0.023^{c}$	$^{A}2.85 \pm 0.023^{c}$	$^{A}2.84 \pm .024^{d}$
T_1S_2	$^{A}2.88 \pm 0.025^{c}$	$^{A}2.87 \pm 0.027^{c}$	$^{A}2.86 \pm .028^{d}$
T_1S_3	$^{A}2.86 \pm 0.023^{c}$	$^{A}2.84 \pm 0.023^{c}$	$^{A}2.82 \pm .023^{d}$
T_1S_4	$^{A}2.84 \pm 0.024^{c}$	$^{A}2.82 \pm 0.024^{c}$	$^{A}2.79 \pm .024^{d}$
T_1S_5	$^{A}2.82 \pm 0.015^{c}$	$^{A}2.79 \pm 0.015^{d}$	$^{A}2.64 \pm .015^{d}$
T_2S_1	$^{A}3.62 \pm 0.015^{b}$	$^{A}3.60 \pm 0.012^{b}$	$^{A}3.59 \pm .012^{c}$
T_2S_2	$^{A}3.61 \pm 0.015^{b}$	$^{A}3.60 \pm 0.009^{b}$	$^{A}3.58 \pm .009^{c}$
T_2S_3	$^{A}3.60 \pm 0.009^{b}$	$^{A}3.58 \pm 0.010^{b}$	$^{A}3.57 \pm .013^{c}$
T_2S_4	$^{A}3.58 \pm 0.009^{b}$	$^{A}3.56 \pm 0.009^{b}$	$^{A}3.54 \pm .009^{c}$
T_2S_5	$^{A}3.56 \pm 0.009^{b}$	$^{A}3.54 \pm 0.009^{b}$	$^{A}3.51 \pm .012^{c}$
T_3S_1	$^{A}4.34 \pm 0.015^{a}$	$^{A}4.33 \pm 0.015^{a}$	$^{A}4.26 \pm .009^{a}$
T_3S_2	$^{A}4.32 \pm 0.022^{a}$	$^{A}4.31 \pm 0.022^{a}$	$^{A}4.04 \pm .334^{b}$
T_3S_3	$^{A}4.30 \pm 0.017^{a}$	$^{A}4.29 \pm 0.017^{a}$	$^{A}4.21 \pm .017^{a}$
T_3S_4	$^{A}4.28 \pm 0.017^{a}$	$^{A}4.25 \pm 0.017^{a}$	$^{A}4.17 \pm .012^{a}$
T_3S_5	$^{A}4.26 \pm 0.006^{a}$	$^{A}4.23 \pm 0.010^{a}$	$^{A}4.16 \pm .012^{a}$
LSD	0.02	0.02	0.089

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table-4.4. Values are expressed as mean ± SE.

A = Mean followed by different superscript alphabets in each row is significantly ($p < 0.05$) different during storage time.

a–d =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples.

**The same letter indicates that not significantly ($p > 0.05$) different.

According to the results presented in table-4.6, mean value of fiber content ranged from 2.80 ± 0.015 to $4.34 \pm 0.015\%$. The data shows that the cakes with prebiotic and

probiotic had a greater percentage of fiber than the control cake, which led to a significance ($p < 0.05$) difference. The highest (4.30 ± 0.017 %) result obtained for T_3S_3 and lowest (2.65 ± 0.019 %) for control sample.

It is clear from the result that fiber content of the cakes were increased with the increasing percentage of inulin. This result was higher than the value of fiber (1.85 to 0.83%) described by Bhat and Bhat (2013) for pumpkin blended cake. Our experimented outcome were similar to that of Sobhy *et al.* (2015) who reported nearly comparable fiber (4.51 ± 0.59 to 1.03 ± 0.27%) for sponge cake incorporated with various levels of jojoba meal and protein isolate. There was not significant ($p > 0.05$) difference in fiber content for all samples together with control sample during storage period.

4.2.4 Firmness (N)

Table-4.7 shows the firmness, with and without prebiotic and probiotic ranged from 2.19 ± 0.006 to 2.95 ± 0.015 (N).The results of analysis revealed that samples were statistically ($p < 0.05$) significant as compared to control sample. Maximum firmness (2.95 ± 0.015N) was reported in T_3S_3 sample, whereas minimum firmness (2.19 ± 0.006N) was observed in control sample at the day of preparation. With the increasing of percentage of inulin, firmness of cake was increased. These results are comparable with the findings of Oliveira *et al.* (2011) who found the similar result for fermented milk. The inverse drift was observed in sweet potato cake reported by Samiha (2015) who explained this increasing tendency due to addition sweet potato in cakes.

Regarding the data, there was a significantly ($p <0.05$) increasing tendency among samples together with control sample throughout the storage period. It is well known in fact that higher microbial growth is one of the causes of a firmness increase in product (Donkor *et al.*, 2007; Tamime, 2005). These results are in accordance with the findings of Oliveira *et al.* (2011) who found the similar result for fermented milk upto 7 days.

Table-4.7: Firmness of cake (N) at different storage periods

Sample	Storage Period		
	0 Day	4 Days	8 Days
Control	$^{B}2.19 \pm 0.006^{k}$	$^{B}2.20 \pm 0.012^{i}$	$^{A}2.36 \pm 0.023^{h}$
T_1S_1	$^{B}2.33 \pm 0.00^{j}$	$^{B}2.61 \pm 0.007^{h}$	$^{A}2.83 \pm 0.023^{fg}$
T_1S_2	$^{B}2.42 \pm 0.008^{i}$	$^{B}2.68 \pm 0.006^{h}$	$^{A}2.88 \pm 0.027^{efg}$
T_1S_3	$^{C}2.51 \pm 0.009^{g}$	$^{B}2.79 \pm 0.006^{g}$	$^{A}2.96 \pm 0.023^{def}$
T_1S_4	$^{B}2.43 \pm 0.009^{i}$	$^{B}2.66 \pm 0.006^{gh}$	$^{A}2.84 \pm 0.024^{fg}$
T_1S_5	$^{B}2.49 \pm 0.006^{h}$	$^{A}2.66 \pm 0.006^{gh}$	$^{A}2.77 \pm 0.015^{g}$
T_2S_1	$^{A}2.63 \pm 0.012^{ef}$	$^{A}2.74 \pm 0.187^{fg}$	$^{A}2.77 \pm 0.012^{g}$
T_2S_2	$^{C}2.69 \pm 0.012^{d}$	$^{B}2.87 \pm 0.006^{ef}$	$^{A}3.04 \pm 0.009^{bcde}$
T_2S_3	$^{C}2.83 \pm 0.009^{b}$	$^{B}2.97 \pm 0.006^{cd}$	$^{A}3.16 \pm 0.010^{abc}$
T_2S_4	$^{A}2.67 \pm 0.007^{de}$	$^{A}2.84 \pm 0.068^{fg}$	$^{A}3.05 \pm 0.009^{bcd}$
T_2S_5	$^{C}2.61 \pm 0.000^{e}$	$^{B}2.79 \pm 0.042^{g}$	$^{A}3.01 \pm 0.009^{cde}$
T_3S_1	$^{B}2.82 \pm 0.015^{c}$	$^{B}2.93 \pm 0.006^{de}$	$^{A}3.18 \pm 0.015^{ab}$
T_3S_2	$^{B}2.86 \pm 0.015^{b}$	$^{A}3.07 \pm 0.015^{b}$	$^{A}3.17 \pm 0.022^{abc}$
T_3S_3	$^{B}2.95 \pm 0.015^{a}$	$^{A}3.14 \pm 0.038^{a}$	$^{A}3.29 \pm 0.017^{a}$
T_3S_4	$^{A}2.82 \pm 0.070^{b}$	$^{A}3.04 \pm 0.015^{b}$	$^{A}3.23 \pm 0.017^{a}$
T_3S_5	$^{B}2.79 \pm 0.034^{b}$	$^{A}3.00 \pm 0.012b^{c}$	$^{A}3.14 \pm 0.010^{abc}$
LSD	0.004	0.19	0.54

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE.

A–C =Means followed by different superscript alphabets in each row are significantly ($p < 0.05$) different during storage time.

a–k =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples.

**Means values in the same column or row showing the same superscript are not significantly different ($p > 0.05$).

4.2.5 Color content

All color data (L^*, a^*, and b^*) corresponding to lightness, redness, and yellowness were expressed in table 4.8 and 4.9 respectively. As shown in table 4.8 and 4.9 crust color of samples was significantly ($P < 0.05$) affected by the addition of inulin and film forming solution. **L* value:**

The L^* value for the control (C) cake were significantly ($p < 0.05$) higher than the L^* values for cake containing prebiotic and probiotic. Moreover, Table-4.8 clearly indicates that control sample attained the highest result (59.59 ± 1.89) while T_3S_5 obtained the lowest (16.80 ± 0.58). Control sample exhibited a light color (white) than others. The similar result was verified by Jun Ho Lee (2015) who got the highest result for control sample (80.10 ± 0.46). Another study was revealed quite similar findings in compared to our results (68.75 ± 1.377 to 57.25 ± 1.652) (Samira *et al.*, 2016).

No significant ($P > 0.05$) differences were found in L* value between the control and treatment samples during storage period.

Table-4.8: L* value of synbiotic cake

Sample	L^*		
	0 Day	4 Days	8 Days
Control	$^{A}59.59 \pm 1.89^{a}$	$^{A}59.97 \pm 0.96^{a}$	$^{A}60.80 \pm 1.22^{a}$
T_1S_1	$^{A}53.74 \pm 0.37^{b}$	$^{A}57.08 \pm 0.63^{b}$	$^{A}55.95 \pm 0.65^{b}$
T_1S_2	$^{A}49.36 \pm 1.09^{c}$	$^{A}50.84 \pm 0.55^{c}$	$^{A}52.63 \pm 0.66^{b}$
T_1S_3	$^{A}49.10 \pm 0.62^{c}$	$^{A}50.58 \pm 1.20^{c}$	$^{A}51.03 \pm 0.60^{b}$
T_1S_4	$^{A}44.45 \pm 1.10^{d}$	$^{A}46.44 \pm 0.67^{d}$	$^{A}46.89 \pm 0.39^{d}$
T_1S_5	$^{A}37.27 \pm 1.31^{f}$	$^{A}42.76 \pm 0.57^{e}$	$^{A}41.30 \pm 1.74^{f}$
T_2S_1	$^{A}47.85 \pm 0.91^{d}$	$^{A}46.78 \pm 1.23^{d}$	$^{A}49.99 \pm 1.74^{c}$
T_2S_2	$^{A}41.93 \pm 0.45^{e}$	$^{A}42.90 \pm 0.38^{e}$	$^{A}44.74 \pm 1.37^{e}$
T_2S_3	$^{A}34.51 \pm 0.46^{g}$	$^{A}40.30 \pm 0.52^{g}$	$^{A}40.92 \pm 1.01^{g}$
T_2S_4	$^{A}29.25 \pm 0.82^{h}$	$^{A}34.98 \pm 0.94^{h}$	$^{A}35.04 \pm 0.83^{h}$
T_2S_5	$^{A}22.88 \pm 0.6^{i}$	$^{A}30.18 \pm 1.00^{j}$	$^{A}28.46 \pm 1.28^{i}$
T_3S_1	$^{A}40.93 \pm 0.87^{e}$	$^{A}42.13 \pm 0.85^{ef}$	$^{A}49.56 \pm 2.66^{c}$
T_3S_2	$^{A}34.15 \pm 0.87^{g}$	$^{A}38.77 \pm 0.9^{i}$	$^{A}44.09 \pm 0.88^{e}$
T_3S_3	$^{A}29.00 \pm 1.25^{h}$	$^{A}35.49 \pm 0.56^{h}$	$^{A}38.23 \pm 0.20^{g}$
T_3S_4	$^{A}22.55 \pm 1.06^{i}$	$^{A}30.08 \pm 0.84^{j}$	$^{A}29.87 \pm 1.18^{i}$
T_3S_5	$^{A}16.80 \pm 0.58^{j}$	$^{A}24.59 \pm 0.48^{k}$	$^{A}25.72 \pm 1.03^{j}$
LSD	3.08	2.09	1.01

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE.

A–B =Means followed by different superscript alphabets in each row are significantly ($p < 0.05$) different during storage time.

a–j =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples.

**Means values in the same column or row showing the same superscripts are not significantly different ($p > 0.05$).

a* value:

The values of a* was mentioned in table -4.9. There was significant difference among the samples (with the control sample and within the formulated samples). Highest value (17.13 ± 0.65) was observed in T_1S_1 sample, whereas lowest for T_1S_5 (3.88 ± 0.37). Regarding the parameters of chromaticity (*a*) the* chromaticity coordinate *a** increased in line with the decreased inulin content and amount of sodium alginate which made the cake more red color. The similar result was cited by Jun Ho lee (2015) for sponge cakes with *Rubus coreanus* powder. Previous study by Fakhreddin *et al* (2016) showed lower result (-2.60 ± 1.0 to 0.55 ± 1.33) of cake with added button mushroom powder. According to Esteller *et al.* (2006), high values for the *a** chroma indicate cakes with a dark red color.

Significant ($P < 0.05$) differences were found in L* value between the control and treatment samples during storage period. Redness (a* value) was increased is all samples during storage.

b* value:

The b* value of formulated cakes were in the range between 43.61 ± 1.97 to 17.85 ± 0.63. The b* value of samples was statistically ($P < 0.05$) significant for the all formulated cakes in this study (table-4.9). This finding was in accordance with the results (33.47 ± 3.70 to 27.72 ± 2.50) observed for cake with added button mushroom powder (Fakhreddin *et al.*, 2016). According to Esteller *et al.* (2006), high values for the *b** parameter translate into samples as strong yellow, which are characteristic of products that have been baked.

There is significant ($P < 0.05$) difference in all samples during storage with control sample. b* value was decreased throughout the storage period.

Table-4.9: a* and b* value of synbiotic cake

Sample	a*			b*		
	0 Day	4 Days	8 Days	0 Day	4 Days	8 Days
Control	$^{B}8.16 \pm 0.87^{d}$	$^{A}11.08 \pm 2.05^{b}$	$^{A}11.77 \pm 2.31^{c}$	$^{A}33.96 \pm 1.03^{b}$	$^{AB}29.59 \pm 0.65^{c}$	$^{B}26.08 \pm 0.99^{c}$
T_1S_1	$^{A}17.13 \pm 0.65^{a}$	$^{A}19.82 \pm 1.30^{a}$	$^{A}21.03 \pm 1.45^{a}$	$^{A}43.61 \pm 1.97^{a}$	$^{A}47.39 \pm 0.79^{a}$	$^{B}45.83 \pm 1.33^{a}$
T_1S_2	$^{B}12.07 \pm 1.26^{c}$	$^{A}14.23 \pm 1.89^{b}$	$^{A}13.78 \pm 0.75^{c}$	$^{A}38.78 \pm 1.11^{b}$	$^{B}34.53 \pm 0.52^{b}$	$^{C}30.58 \pm 1.22^{b}$
T_1S_3	$^{B}9.19 \pm 1.55^{d}$	$^{A}10.07 \pm 0.79^{b}$	$^{A}10.56 \pm 0.42^{c}$	$^{A}33.34 \pm 0.87^{b}$	$^{A}30.27 \pm 1.37^{c}$	$^{B}25.89 \pm 1.48^{c}$
T_1S_4	$^{B}7.37 \pm 0.23^{d}$	$^{B}7.21 \pm 0.25^{b}$	$^{A}8.11 \pm 0.72^{c}$	$^{A}28.37 \pm 1.10^{c}$	$^{B}22.99 \pm 0.93^{e}$	$^{C}18.92 \pm 0.92^{d}$
T_1S_5	$^{C}3.88 \pm 0.37^{e}$	$^{AB}3.90 \pm 0.90^{b}$	$^{A}4.21 \pm 0.63^{c}$	$^{A}22.21 \pm 0.92^{c}$	$^{B}18.45 \pm 1.05^{e}$	$^{C}17.17 \pm 0.97^{d}$
T_2S_1	$^{A}16.96 \pm 0.92^{a}$	$^{A}19.03 \pm 1.10^{a}$	$^{A}19.13 \pm 0.27^{c}$	$^{A}34.20 \pm 1.07^{b}$	$^{B}30.18 \pm 1.06^{c}$	$^{B}27.37 \pm 0.28^{c}$
T_2S_2	$^{B}14.69 \pm 0.75^{b}$	$^{BC}14.80 \pm 0.82^{b}$	$^{A}15.10 \pm 0.96^{c}$	$^{A}28.43 \pm 1.81^{c}$	$^{B}24.02 \pm 0.85^{de}$	$^{C}20.28 \pm 0.76^{d}$
T_2S_3	$^{B}11.97 \pm 0.76^{c}$	$^{A}12.28 \pm 1.01^{b}$	$^{A}12.56 \pm 1.35^{c}$	$^{A}22.17 \pm 0.93^{c}$	$^{A}20.08 \pm 0.98^{e}$	$^{C}15.70 \pm 0.68^{d}$
T_2S_4	$^{B}8.70 \pm 0.33^{d}$	$^{A}9.20 \pm 1.35^{b}$	$^{A}10.81 \pm 0.73^{c}$	$^{A}19.08 \pm 0.86^{c}$	$^{B}18.89 \pm 0.31^{e}$	$^{C}15.15 \pm 0.84^{d}$
T_2S_5	$^{B}4.24 \pm 0.89^{e}$	$^{B}4.75 \pm 1.09^{b}$	$^{A}5.19 \pm 1.07^{c}$	$^{A}14.84 \pm 0.71^{c}$	$^{B}16.42 \pm 0.35^{h}$	$^{C}10.95 \pm 0.20^{e}$
T_3S_1	$^{B}12.64 \pm 0.79^{bc}$	$^{A}14.07 \pm 1.07^{b}$	$^{A}14.29 \pm 0.34^{c}$	$^{A}26.55 \pm 1.42^{c}$	$^{A}24.20 \pm 1.24^{d}$	$^{B}24.88 \pm 1.12^{c}$
T_3S_2	$^{B}10.96 \pm 0.46^{cd}$	$^{A}11.20 \pm 0.75^{b}$	$^{A}11.52 \pm 0.66^{c}$	$^{A}23.32 \pm 2.26^{c}$	$^{A}20.58 \pm 0.66^{e}$	$^{A}20.71 \pm 0.73^{d}$
T_3S_3	$^{B}8.79 \pm 0.46^{def}$	$^{AB}9.04 \pm 0.43^{b}$	$^{A}9.57 \pm 0.56^{c}$	$^{A}20.68 \pm 1.64^{c}$	$^{B}18.70 \pm 0.91^{e}$	$^{B}17.70 \pm 0.69^{d}$
T_3S_4	$^{B}6.75 \pm 0.32^{fg}$	$^{B}6.41 \pm 0.66^{b}$	$^{A}7.01 \pm 0.98^{c}$	$^{A}18.88 \pm 0.91^{c}$	$^{A}17.54 \pm 1.17^{e}$	$^{B}14.69 \pm 0.66^{d}$
T_3S_5	$^{C}4.59 \pm 0.42^{e}$	$^{B}4.73 \pm 0.74^{b}$	$^{A}5.12 \pm 0.5^{c}$	$^{A}15.85 \pm 0.63^{c}$	$^{B}16.89 \pm 0.11^{e}$	$^{B}10.22 \pm 1.07^{e}$
LSD	2.18	3.41	2.69	4.39	2.76	4.03

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE. A =Mean followed by different superscript alphabets in each row are significantly ($p < 0.05$) different during storage time. a–n =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples. **Means values in the same column or row showing the same superscripts are not significantly different ($p > 0.05$).

Table-4.10: Viable count of synbiotic cake

Sample	Storage Period					
	0 Day		4 Days		8 Days	
	Before *In vitro* log CFU / g of crust	After *In vitro* log CFU / g of crust	Before *In vitro* log CFU / g of crust	After *In vitro* log CFU / g of crust	Before *In vitro* log CFU / g of crust	After *In vitro* log CFU / g of crust
Control	$^{e}1.477 \pm .002^{A}$	$^{c}1.301 \pm .000^{A}$	$^{e}2.523 \pm .002^{A}$	$^{d}2.337 \pm .000^{A}$	$^{c}3.865 \pm .001^{A}$	$^{b}3.724 \pm .001^{A}$
T_1S_1	$^{abcd}7.793 \pm .012^{A}$	$^{ab}6.782 \pm .094^{A}$	$^{c}7.846 \pm .286^{A}$	$^{c}7.808 \pm .338^{A}$	$^{ab}8.888 \pm .286^{A}$	$^{a}7.814 \pm .338^{A}$
T_1S_2	$^{abcd}7.798 \pm .026^{A}$	$^{ab}6.783 \pm .157^{A}$	$^{bcd}7.859 \pm .146^{A}$	$^{bc}7.824 \pm .371^{A}$	$^{ab}8.895 \pm .146^{A}$	$^{a}7.823 \pm .371^{A}$
T_1S_3	$^{ab}7.814 \pm .225^{A}$	$^{ab}6.804 \pm .297^{A}$	$^{ab}7.871 \pm .338^{A}$	$^{abc}7.847 \pm .333^{A}$	$^{ab}8.914 \pm .338^{A}$	$^{a}7.857 \pm .333^{A}$
T_1S_4	$^{bcd}7.794 \pm .012^{A}$	$^{ab}6.771 \pm .264^{A}$	$^{b}7.851 \pm .068^{A}$	$^{bc}7.833 \pm .404^{A}$	$^{ab}8.892 \pm .068^{A}$	$^{a}7.832 \pm .404^{A}$
T_1S_5	$^{bcd}7.766 \pm .003^{A}$	$^{b}6.751 \pm .252^{A}$	$^{d}7.834 \pm .300^{A}$	$^{bc}7.824 \pm .273^{A}$	$^{b}8.879 \pm .300^{A}$	$^{a}7.818 \pm .273^{A}$
T_2S_1	$^{abcd}7.790 \pm .026^{A}$	$^{ab}6.748 \pm .280^{A}$	$^{abcd}7.869 \pm .075^{A}$	$^{bc}7.837 \pm .325^{A}$	$^{ab}8.904 \pm .075^{A}$	$^{a}7.850 \pm .325^{A}$
T_2S_2	$^{abcd}7.800 \pm .078^{A}$	$^{ab}6.786 \pm .259^{A}$	$^{abcd}7.877 \pm .024^{A}$	$^{bc}7.838 \pm .330^{A}$	$^{ab}8.919 \pm .024^{A}$	$^{a}7.853 \pm .330^{A}$
T_2S_3	$^{ab}7.814 \pm .230A$	$^{ab}6.784 \pm .193^{A}$	$^{abcd}7.896 \pm .043^{A}$	$^{bc}7.871 \pm .349^{A}$	$^{ab}8.923 \pm .043^{A}$	$^{a}7.873 \pm .349^{A}$
T_2S_4	$^{abc}7.806 \pm .166^{A}$	$^{ab}6.774 \pm .358^{A}$	$^{abcd}7.873 \pm .064^{A}$	$^{bc}7.836 \pm .335^{A}$	$^{ab}8.908 \pm .064^{A}$	$^{a}7.855 \pm .335^{A}$
T_2S_5	$^{d}7.792 \pm .258^{A}$	$^{ab}6.768 \pm .050^{A}$	$^{bcd}7.860 \pm .009^{A}$	$^{bc}7.831 \pm .322^{A}$	$^{ab}8.894 \pm .009^{A}$	$^{a}7.863 \pm .322^{A}$
T_3S_1	$^{abcd}7.794 \pm .015^{A}$	$^{ab}6.781 \pm .103^{A}$	$^{abcd}7.874 \pm .329^{A}$	$^{ab}7.863 \pm .075^{A}$	$^{ab}8.913 \pm .329^{A}$	$^{a}7.866 \pm .075^{A}$
T_3S_2	$^{c}7.778 \pm .217^{A}$	$^{ab}6.710 \pm .065^{A}$	$^{abcd}7.878 \pm .309^{A}$	$^{ab}7.871 \pm .024^{A}$	$^{ab}8.916 \pm .309^{A}$	$^{a}7.871 \pm .024^{A}$
T_3S_3	$^{a}7.823 \pm .205^{A}$	$^{a}6.791 \pm .273^{A}$	$^{a}7.910 \pm .420^{A}$	$^{a}7.896 \pm .043^{A}$	$^{a}8.933 \pm .420^{A}$	$^{a}7.877 \pm .043^{A}$
T_3S_4	$^{abc}7.820 \pm .148^{A}$	$^{ab}6.802 \pm .917^{A}$	$^{abcd}7.897 \pm .307^{A}$	$^{ab}7.873 \pm .064^{A}$	$^{ab}8.915 \pm .307^{A}$	$^{a}7.853 \pm .064^{A}$
T_3S_5	$^{abcd}7.792 \pm .079^{A}$	$^{b}6.747 \pm .252^{A}$	$^{abc}7.885 \pm .302^{A}$	$^{abc}7.860 \pm .009^{A}$	$^{ab}8.910 \pm .302^{A}$	$^{a}7.860 \pm .009^{A}$
LSD	0.14	0.32	0.24	0.28	0.30	0.40

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE. A =Mean followed by different superscript alphabets in each row are significantly ($p < 0.05$) different during storage time. a–n =Means followed by different superscript alphabets in each column are significantly ($p < 0.05$) different among samples. **Means values in the same column or row showing the same superscripts are not significantly different ($p > 0.05$).

4.2.6 Viable cell count

The symbiotic cake samples were microbiologically tested from 3 h after baking to 8 days storage in order to evaluate the retention of probiotic viability in ambient temperature. Microbiological analysis conducted for developed cakes were revealed that a significant ($P < 0.05$) difference was observed among sample with compare to control sample (Table-4.10). The highest result (7.823 log cfu g^{-1}) was found in T_3S_3 whereas the lowest result (1.477 log cfu g^{-1}) found for control sample. All the symbiotic cakes were significantly ($p < 0.05$) different from control sample in both case of before and after digestion. After *In vitro* digestion, these viability were slightly decreased varied from 1.301 to 6.791 log cfu g^{-1}.

Viability count of all the samples including control, did not significantly ($P > 0.05$) throughout the storage upto 8 days (table -4.10). However, these results indicate good maintenance of viability in products for the probiotic microorganism to grow during storage period. Populations were above 6-8 log cfu g^{-1} during the whole shelf life of the product in case of before and after digestion. According to WHO (2006), the recommended level of probiotic microorganisms in food at the time of consumption is > 6-7 logcfu g^{-1} which have beneficial effects on the consumer's health. Which consequently approve the synbiotic cake developed in the present study as a potential vehicle for the *Lactobacillus spp.* Other authors also reported satisfactory probiotic viability (Buriti *et al.*, 2005a; Gomes & Malcata, 1999; Vinderola *et al.*, 2000), confirming the use of fresh cheeses like petit-suisse cheese as vehicles for probiotics.

4.2.7 Sensory acceptance

The objective of most quantitative consumer research conducted in support of product development is to determine consumers' affective reaction to new or revised products. The level of consumer acceptance is often assessed by asking consumers to rate how much they like a product overall, using a nine-point hedonic scale (Popper *et al.*, 2004). It is known that affective tests (like acceptability tests) help to answer whether the product has commercial potential, especially when consumers are used as panelists.

Texture:

Sensorial results of texture are presented in Fig 1. Regarding texture, all samples showed significant ($P<0.05$) difference. T_3S_3 (8.37) had the highest average score while control

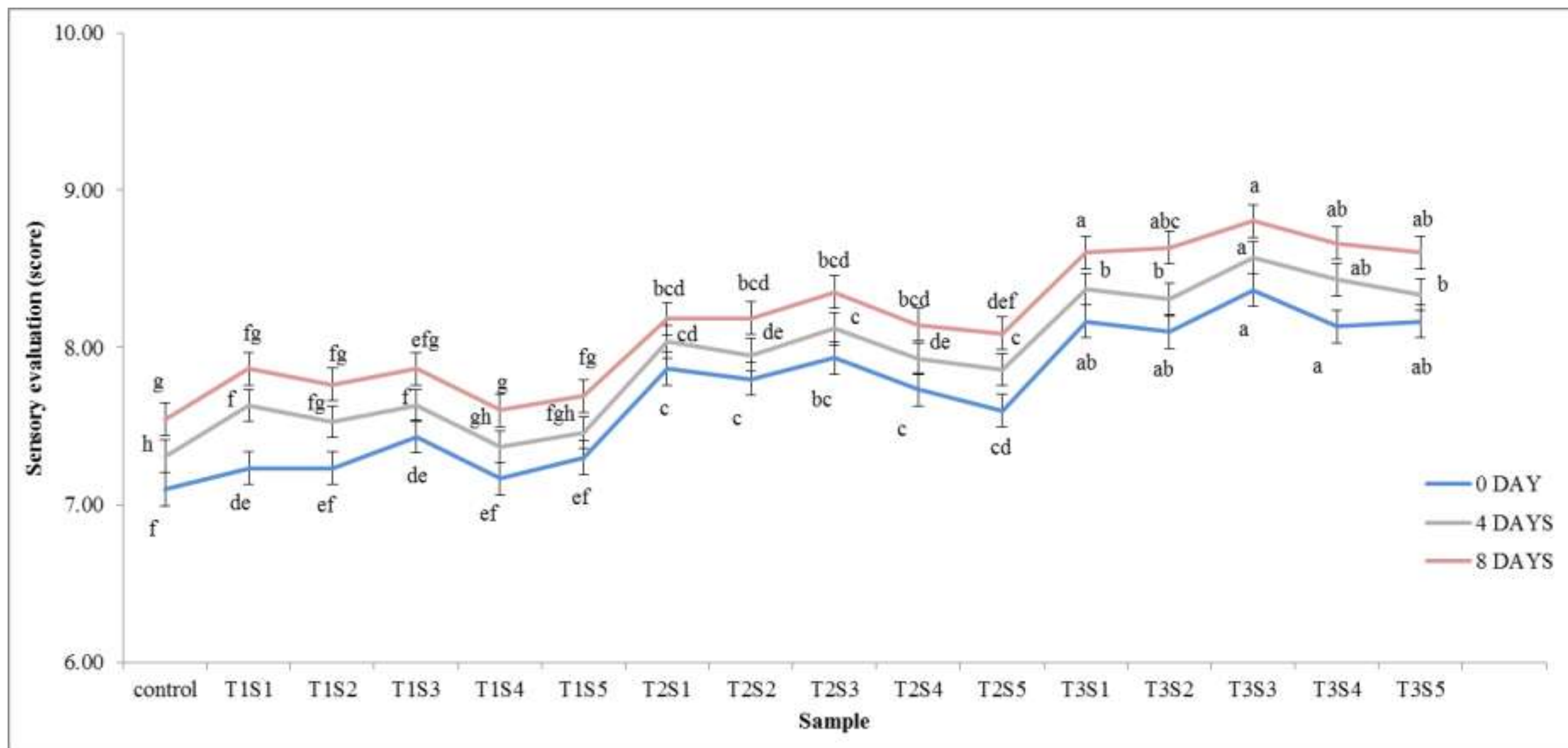


Fig. 4.1: Effect of inulin and *Lactobacillus spp.* on texture of synbiotic cakes with control sample and storage study

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table-4.4. Values are expressed as mean ± SE. a–h (lowercase) Means followed by different superscript alphabets are significantly different among samples ($p < 0.05$).

(7.10) had lowest score. The formulations with 3% inulin attained highest score in terms of texture that may have been associated with the increasing inulin percentage. Tungland and Meyer (2002) reported that concentrations of inulin can increase the structure and texture acceptance of product. This behavior was similar to Buriti (2005) who reported that presence of inulin improved the texture of fresh cream cheese with *Lactobacillus paracasei*.

Significance ($P<0.05$) increase was observed during storage period. It is clear from the result that texture of the cakes were increased with the increasing percentage of inulin. Another possible explanation for these textural results may be the growth of *Lactobacillus spp.* during storage period (Oliveira *et al.*, 2011).

Crust brownness:

A significant difference ($p < 0.05$) among sample was observed (Fig 4.2). The results obtained in the sensory analysis showed that, in general, all the formulations received scores between 6.53 and 8.50. Control sample differed from others by presenting highest score (8.50) whereas T_3S_2 (6.53) showed to have lowest one. This variation might be related to the addition of inulin in cakes. These results are in good agreement with Sabanis *et al.* (2006) who found that organoleptic property (crust color) were obtained poor marks with the increasing percentage of of chickpea flour. Similar findings were found by Sheikholeslami *et al.* (2015) who added glycerol to Barbari bread and found good crust brown color.

Regarding crust brownness, there is a significant ($p < 0.05$) difference for all samples together with control sample during storage time. These results are accordance with the finding given by Al-Sayed and Ahmed (2013) who found decreasing tendency in case of crust color for watermelon rinds cake throughout the storage period.

Crumb brownness:

The results for crumb brownness obtained in the sensory analysis are summarized in fig 3.As shown in fig 3, it is clearly stated that there is significant ($p < 0.05$) difference among samples. Control sample (8.13) scored the highest average score whereas T_3S_2 (6.20) obtained scored lowest score for crumb brownness. Five formulations (T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5) with 3% inulin had lowest acceptability, a factor that may have been associated with the slightly dark color caused by the adding greater percentage of

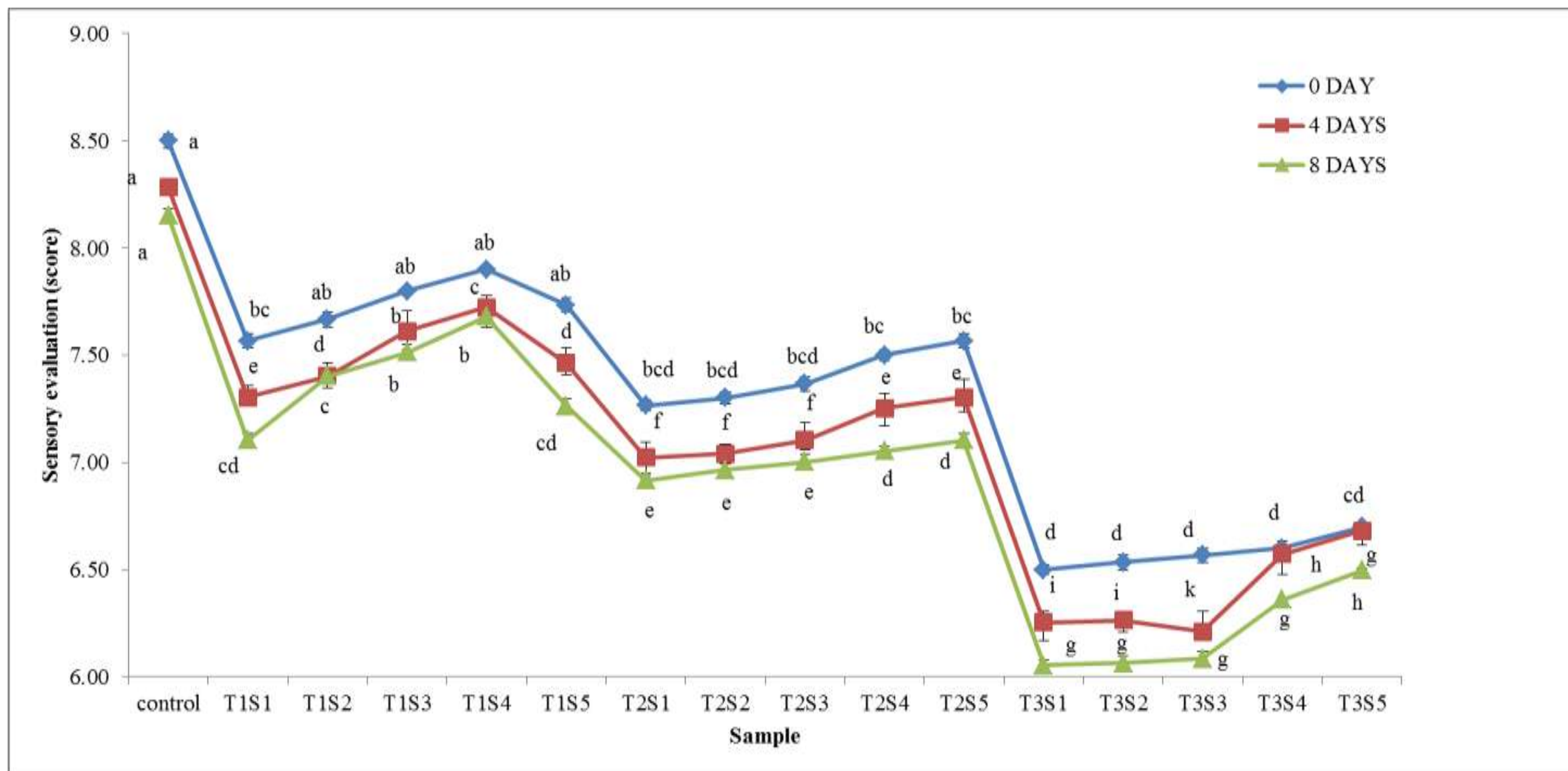


Fig. 4.2: Effect of inulin and *Lactobacillus spp.* on crust color of synbiotic cakes with control sample and storage study

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE. a–i (lowercase) Means followed by different superscript alphabets are significantly different among samples ($p < 0.05$).

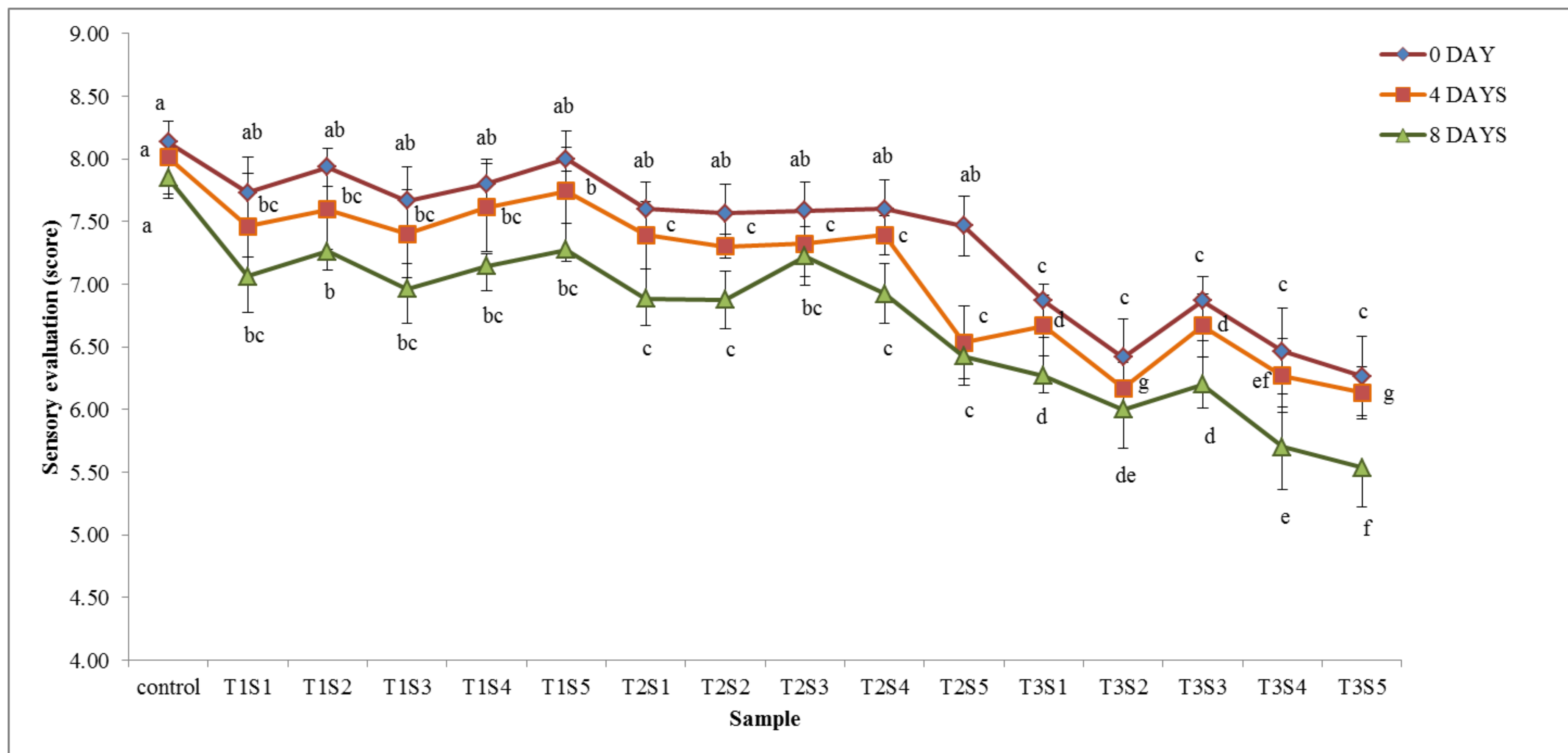


Fig. 4.3: Effect of inulin and *Lactobacillus spp.* on crumb color of synbiotic cakes with control sample and storage study

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE. a–g (lowercase) Means followed by different superscript alphabets are significantly different among samples ($p < 0.05$).

inulin. Similar results were stated by Silanikove *et al.* (2006) in relation to cakes developed with increasing percentages of carob flour.

Significance ($P<0.05$) decrease was observed during storage period. These results are in accordance with the finding given by Al-Sayed and Ahmed (2013) who found decreasing tendency in case of crust color for watermelon rinds cake throughout the storage period.

Flavor:

Regarding flavor, the formulation received scores from 7.07 to 5.00 and differed significantly ($p < 0.05$) from each other. Among all samples, control sample (7.07) showed to have highest value while T3S3 showed to have lowest one. Cakes containing 1% inulin (T_1S_1, T_1S_2, T_1S_3, T_1S_4, and T_1S_5) were scored well as compared to control sample. Cakes containing 3% inulin (T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5) did not perform well and obtained poor marks. Possibly the presence of highest amount of inulin contributed to the unfavorable performance in case of T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5. This could be affected by the impact of ginger flavor on the cake. A number of authors reported that probiotic microorganisms affected the flavor of the food product to which they were added. Bernardi *et al.,* (2004) observed that the products supplemented with probiotic bacteria had a moderate acceptance, whereas ice-cream not supplemented with these microorganisms was well accepted by consumers. Throughout the accelerated storage, there were significant differences ($p < 0.05$) in flavor among synbiotic samples as well as control sample. A decreasing tendency was observed for all samples during storage time.

Taste:

Figure 5 demonstrates the taste of the synbiotic cake with control sample. A significant difference ($p < 0.05$) was noted among the samples. The highest value (8.73) was observed for control sample when lowest value was 4.77 for T_3S_4. Cakes containing 2% inulin (T_2S_1, T_2S_2, T_2S_3, T_2S_4, and T_2S_5) were considered most preferred trail especially T2S3. Cakes containing 3% inulin (T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5) did not perform well and obtained poor marks. Possibly the presence of highest amount of inulin contributed to the lowest taste in case of T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5. Regarding taste, there is a significant ($p < 0.05$) decrease for all samples together with control sample during storage time. These results are in good agreement with Al-Sayed and Ahmed (2013) who found declining tendency in case of taste for watermelon rinds cake during the storage period.

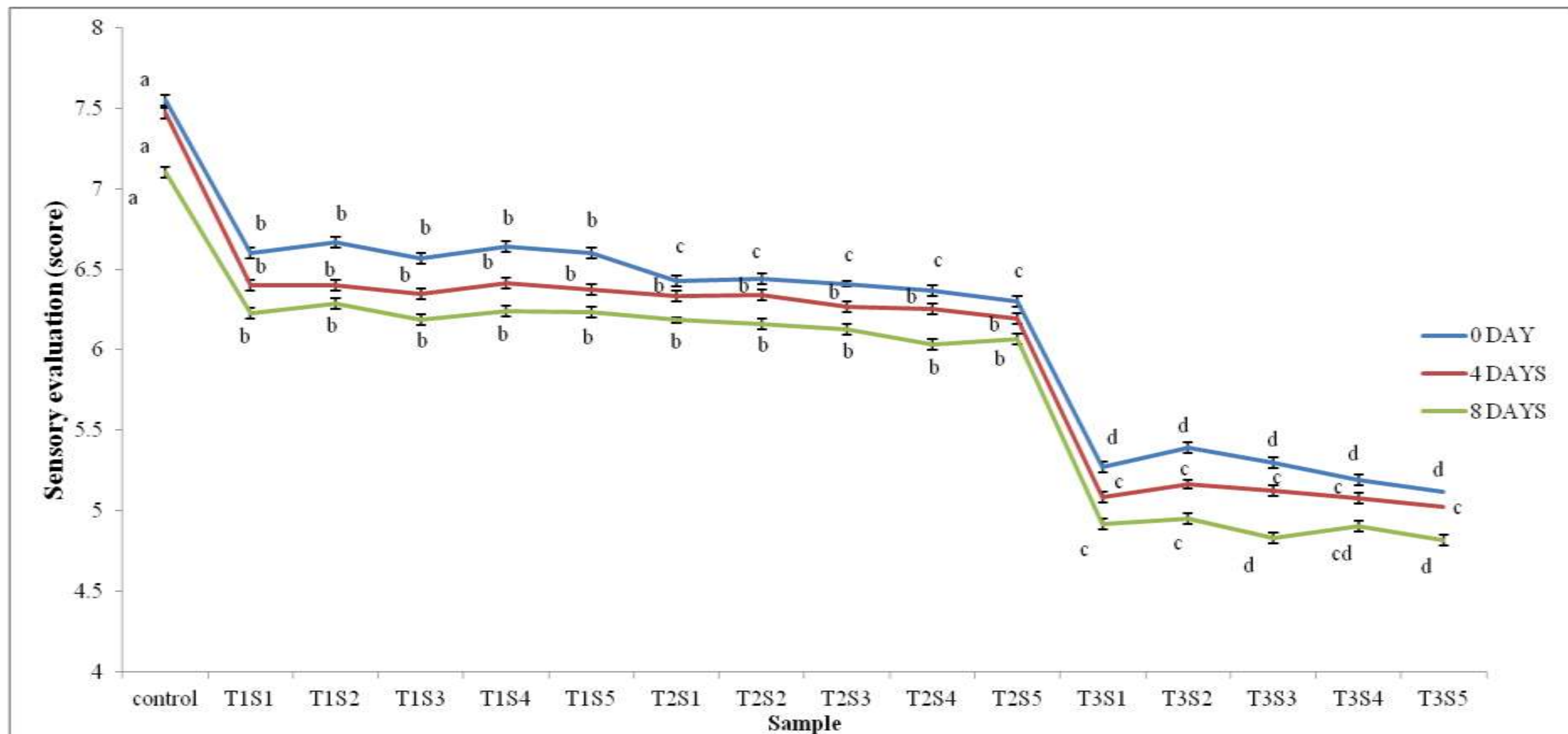


Fig. 4.4: Effect of inulin and *Lactobacillus spp.* on flavor of synbiotic cakes with control sample and storage study

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table -4.4. Values are expressed as mean ± SE. a–d (lowercase) Means followed by different superscript alphabets are significantly different among samples ($p < 0.05$).

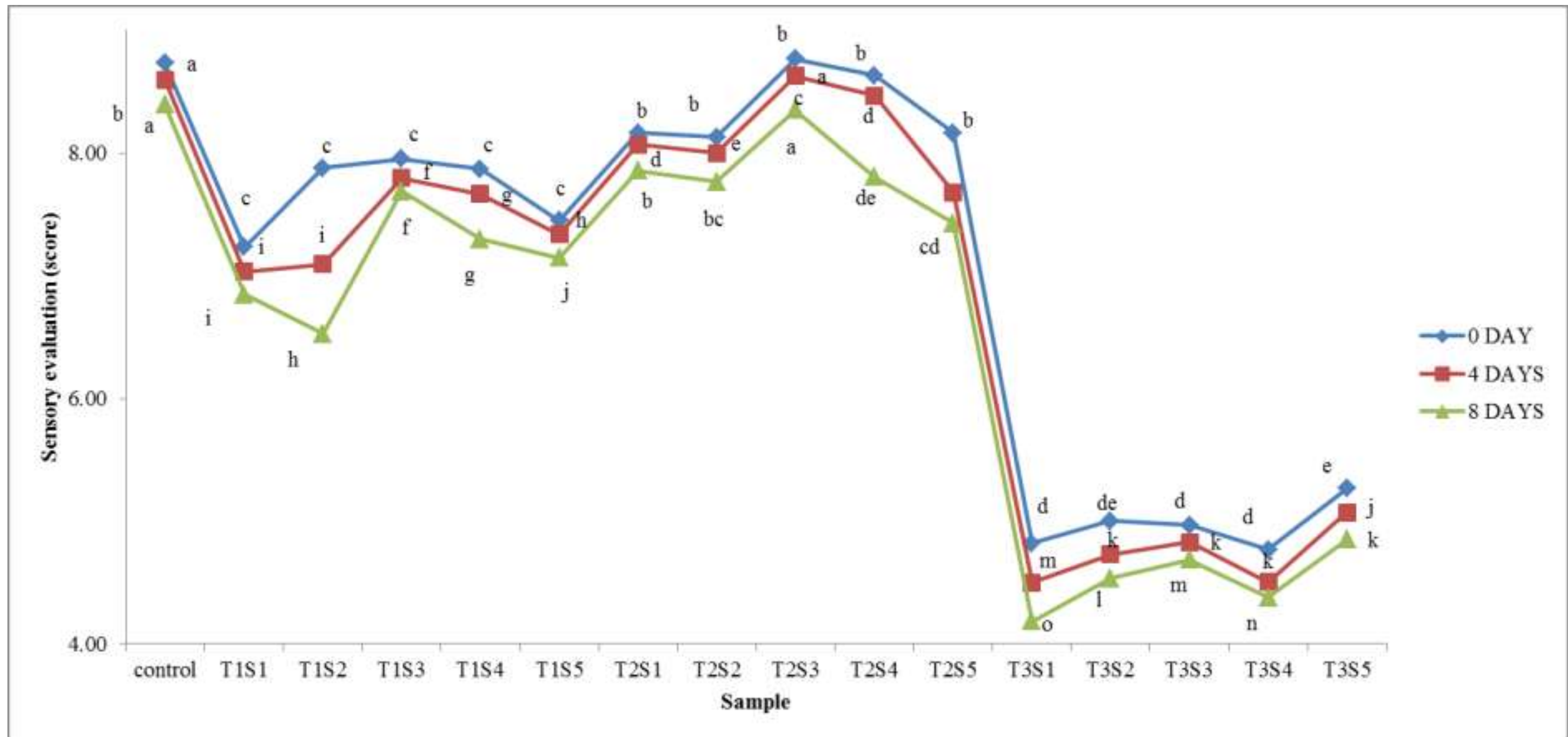


Fig. 4.5: Effect of inulin and *Lactobacillus spp.* on taste of synbiotic cakes with control sample and storage study

Control, T_1S_1, T_1S_2, T_1S_3, T_1S_4, T_1S_5, T_2S_1, T_2S_2, T_2S_3, T_2S_4, T_2S_5, T_3S_1, T_3S_2, T_3S_3, T_3S_4 and T_3S_5 abbreviations are given in Table-4.4. Values are expressed as mean ± SE. a–o (lowercase) Means followed by different superscript alphabets are significantly different among samples ($p < 0.05$).

CHAPTER V

SUMMARY AND CONCLUSION

The potentially synbiotic cake obtained in the present study turned out to be feasible vehicles for probiotic and prebiotic ingredients. Physico-chemical properties of inulin extracted from garlic were found to be suitable for use in a wide range of food applications. Cakes containing 3% inulin (T_3) was found to be superior in terms of fiber, firmness and viability of bacteria but regarding taste, T_3 formulation had the worst performance throughout the storage period. On the contrary cakes containing 2% inulin especially T_2S_3 sample exhibited the best result in terms of sensory attributes especially taste. Viable count of all the cakes were in acceptable limit (>6- 7 logcfu g^{-1}) upto 8 days of storage. Therefore prepared cake T_2S_3 (2% inulin + 50:50 film forming solution) seems to be the most promising concerning the simultaneous achievement of inulin and viable count of *Lactobacillus spp* and sensory acceptance.

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APPENDICES

Appendix I: Analysis of variance (ANOVA) for moisture, pH, fiber, firmness (0 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Moisture	Between Groups	262.848	15	17.523	105.244	
	Within Groups	5.328	32	0.166		0.244
	Total	268.176	47			
pH	Between Groups	0.296	15	0.020	631.449	
	Within Groups	0.001	32	0.000031		0.005
	Total	0.297	47			
Fiber	Between Groups	18.380	15	1.225	1349.015	
	Within Groups	0.029	32	0.001		
	Total	18.409	47			0.02
Firmness	Between Groups	0.973	15	0.065	73.064	
	Within Groups	0.028	32	0.001		0.004
	Total	1.001	47			

Significant at 5% level of significance

Appendix II: Analysis of variance (ANOVA) for moisture, pH, fiber, firmness (4 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Moisture	Between Groups	279.820	15	18.655	218.343	
	Within Groups	2.734	32	.085		
	Total	282.554	47			0.553
pH	Between Groups	.411	15	.027	2631.667	
	Within Groups	.000337	32	.000010		0.008
	Total	.412	47			
Fiber	Between Groups	18.343	15	1.223	1304.427	
	Within Groups	.030	32	.001		0.02
	Total	18.373	47			
Firmness	Between Groups	.863	15	.058	61.397	
	Within Groups	.030	32	.001		0.19
	Total	.893	47			

Significant at 5% level of significance

Appendix III: Analysis of variance (ANOVA) for moisture, pH, fiber, firmness (8 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Moisture	Between Groups	271.614	15	18.108	152.748	
	Within Groups	3.793	32	.119		0.207
	Total	275.408	47			
pH	Between Groups	19.732	15	1.315	70158.393	
	Within Groups	.001	32	.000019		0.003
	Total	19.733	47			
Fiber	Between Groups	17.171	15	1.145	52.759	
	Within Groups	.694	32	.022		0.089
	Total	17.866	47			
Firmness	Between Groups	.908	15	.061	62.885	
	Within Groups	.031	32	.001		0.54
	Total	.939	47			

Significant at 5% level of significance

Appendix IV: Analysis of variance (ANOVA) for microbial viability (0 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Before *In vitro*	Between Groups	111.890	15	7.459	123.074	
	Within Groups	1.939	32	.061		0.14
	Total	113.829	47			
After *In vitro*	Between Groups	106.865	15	7.124	24.317	
	Within Groups	9.375	32	.293		0.32
	Total	116.241	47			

Significant at 5% level of significance

Appendix V: Analysis of variance (ANOVA) for microbial viability (4 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
A.I.V	Between Groups	161.279	15	10.752	64.486	
	Within Groups	5.335	32	.167		0.24
	Total	166.615	47			
B.I.V	Between Groups	146.795	15	9.786	44.768	
	Within Groups	6.995	32	.219		0.28
	Total	153.790	47			

Significant at 5% level of significance

Appendix VI: Analysis of variance (ANOVA) for microbial viability (8 day)

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
A.I.V	Between Groups	186.250	15	12.417	50.015	
	Within Groups	7.944	32	.248		0.30
	Total	194.194	47			
B.I.V	Between Groups	146.934	15	9.796	21.605	
	Within Groups	14.509	32	.453		0.40
	Total	161.443	47			

Significant at 5% level of significance

Appendix VII: Analysis of variance (ANOVA) for color (L*, a* and b*) at 0 day

Source of Variance		Sum of Squares	Df	Mean Square	F	LSD
Color L	Between Groups	6682.542	15	445.503	157.746	
	Within Groups	90.374	32	26.472		3.08
	Total	6772.916	47			
Color A	Between Groups	726.404	15	48.427	26.961	
	Within Groups	57.478	32	13.25		2.18
	Total	783.882	47			
Color B	Between Groups	4548.314	15	303.221	56.518	
	Within Groups	171.681	32	53.65		4.39
	Total	4719.995	47			

Significant at 5% level of significance

Appendix VIII: Analysis of variance (ANOVA) for color (L*, a* and b*) at 4 day

Source of Variance		Sum of Squares	Df	Mean Square	F	LSD
Color L	Between Groups	4307.577	15	287.172	146.933	
	Within Groups	62.542	32	12.14		2.09
	Total	4370.119	47			
Color A	Between Groups	1069.412	15	256.12	22.101	3.41
	Within Groups	103.227	32	33.49		
	Total	1172.638	47			
Color B	Between Groups	3607.925	15	240.528	104.065	
	Within Groups	73.963	32	21.27		2.76
	Total		47			

Significant at 5% level of significance

Appendix IX: Analysis of variance (ANOVA) for color (L*, a* and b*) at 8 day

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Color L	Between Groups	4307.577	15	287.172	146.933	
	Within Groups	62.542	32	2.89		1.01
	Total	4370.119	47			
Color A	Between Groups	1069.412	15	71.294	22.101	
	Within Groups	103.227	32	14.65		2.69
	Total	1172.638	47			
Color B	Between Groups	3607.925	15	240.528	104.065	
	Within Groups	73.963	32	45.13		4.03
	Total	3681.888	47			

Significant at 5% level of significance

Appendix X: Analysis of variance (ANOVA) for sensory characteristics at 0 day

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Texture	Between Groups	118.800	15	7.920	75.173	
	Within Groups	23.600	224	0.41		0.12
	Total	142.400	239			
Crust Brownness	Between Groups	43.396	15	2.893	26.705	
	Within Groups	24.267	224	0.311		0.28
	Total	67.663	239			
Crumb Color	Between Groups	47.733	15	3.182	61.450	
	Within Groups	11.600	224	.830		0.15
	Total	59.333	239			
Flavor	Between Groups	54.867	15	3.658	438.933	
	Within Groups	1.867	224	.0.30		0.15
	Total	56.733	239			
Taste	Between Groups	500.929	15	33.395	1144.981	
	Within Groups	6.533	224	.0.001		0.18
	Total	507.463	239			

Significant at 5% level of significance

Appendix XI: Analysis of variance (ANOVA) for sensory characteristics at 0 day

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Texture	Between Groups	16.375	15	1.931	.025	
	Within Groups	81.400	224	0.009		0.055
	Total	97.775	239			
Crust Brownness	Between Groups	15.477	15	1.032	459.421	
	Within Groups	.072	224	.02		0.08
	Total	15.549	239			
Crumb Color	Between Groups	19.101	15	1.273	45.614	
	Within Groups	.893	224	.033		0.12
	Total	19.994	239			
Flavor	Between Groups	19.745	15	1.316	16.038	
	Within Groups	2.626	224	.390		0.37
	Total	22.372	239			
Taste	Between Groups	115.922	15	7.728	4823.806	
	Within Groups	.051	224	.002		0.026
	Total	115.973	239			

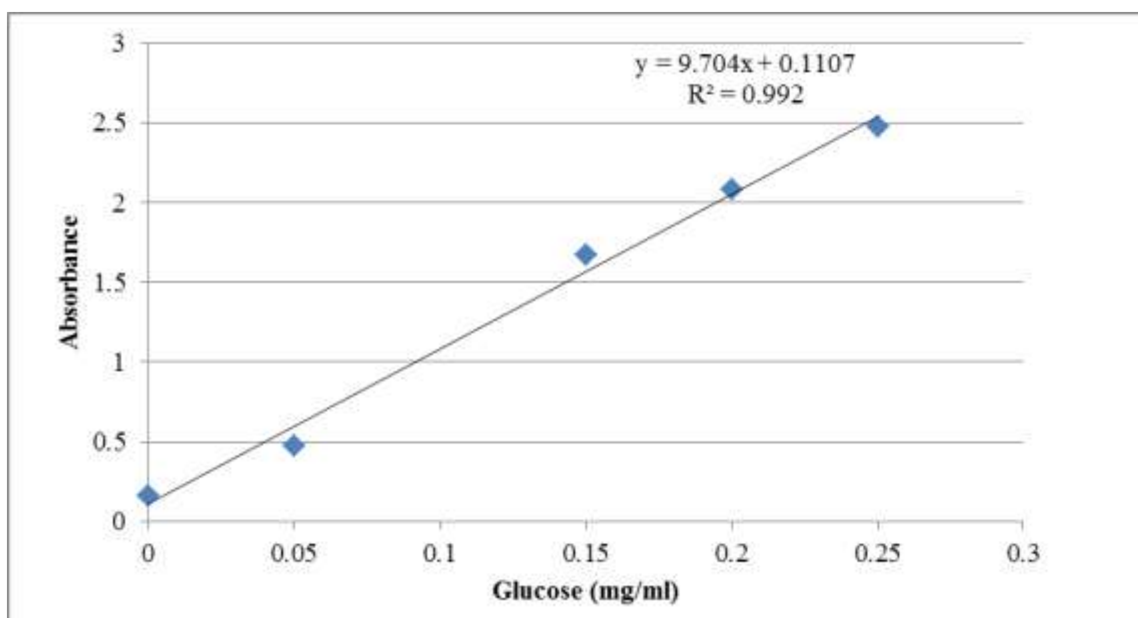
Significant at 5% level of significance

Appendix XII: Analysis of variance (ANOVA) for sensory characteristics at 0 day

Source of Variance		Sum of Squares	df	Mean Square	F	LSD
Texture	Between Groups	15.494	15	1.033	2.320	
	Within Groups	64.100	224	0.27		0.34
	Total	79.594	239			
Crust Brownness	Between Groups	15.549	15	1.074	219.797	
	Within Groups	16.107	224	.005		0.041
	Total	.156	239			
Crumb Color	Between Groups	22.877	15	1.525	29.550	
	Within Groups	1.652	224	.61		0.14
	Total	24.528	239			
Flavor	Between Groups	21.908	15	1.461	21.013	
	Within Groups	2.224	224	.277		0.30
	Total	24.132	239			
Taste	Between Groups	97.881	15	6.525	2088.132	
	Within Groups	.100	224	.003		0.03
	Total	97.981	239			

Significant at 5% level of significance

Appendix XIII: Standard curve for total sugar



Appendix XIV: Standard curve for reducing sugar