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Effects of Yogurt Fortification with Different Legumes Protein on the Physio-Chemical, Microbiological, and Rheological Properties

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EFFECTS OF YOGURT FORTIFICATION WITH DIFFERENT LEGUMES PROTEIN ON THE PHYSIO-CHEMICAL, MICROBIOLOGICAL, AND RHEOLOGICAL PROPERTIES

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The thesis is approved as a creditable and independent investigation by candidate for the Master of Science degree and is acceptable for meeting the thesis requirement for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

K	:	Consistency Index
KBPC	:	Kidney Bean Protein Concentrate
n	:	Flow Behavior Index
PBPC	:	Pinto Bean Protein Concentrate
TA	:	Titratable Acidity
TS	:	Total Solids
TSC	:	Total Solids Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

EFFECTS OF YOGURT FORTIFICATION WITH DIFFERENT LEGUMES PROTEIN ON THE PHYSIO-CHEMICAL, MICROBIOLOGICAL, AND RHEOLOGICAL PROPERTIES

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Nowadays, yogurt (a nutritious fermented dairy product) is getting popular around all over the world due to beneficial action provided by bacteria present in yogurt culture. Along with that, diet plans, which are high in protein and low in fat, has become appealing for growing healthy conscious population that makes a huge shift toward plant-based protein consumption. Therefore, the main objective of this research was to study the effects of yogurt fortification with extracted protein of different beans (legumes)- pinto and kidney beans on the physio-chemical (Moisture, Fat, Protein, Ash, Total Solids Content (TSC), pH, Titratable Acidity (TA), Water Holding Capacity (WHC), and Color), microbiological, and rheological properties. The main reason for using legumes was that they are an excellent source of protein with miscellaneous applications in the food industry.

Protein was extracted from the beans by following the isoelectric point precipitation method. Protein concentrate was added to the yogurt at different fortification levels of 0, 2.5, 5, 7.5, and 10% before the inoculation stage. 0% fortification represents the control yogurt sample. The fortified and control milk samples were incubated at 42±1 °C until the pH reached 4.6. The prepared control and fortified yogurt samples were stored at 5^{o}C and analyzed for their physio-chemical, microbiological and

rheological properties over a storage period of 1, 7, 14, 21 and 28 days respectively. Physio-chemical properties were determined by standard procedure (AOAC), microbiological by total plate procedure and power law model used to describe the rheological behavior of the control and fortified yogurt.

The ANOVA procedures applied on physiochemical properties of control and both pinto bean protein concentrate (PBPC) and kidney bean protein concentrate (KBPC) fortified yogurt were found to be statistically significant with p-value less than 0.05 and R^2 values to be more than 0.93 for all the physiochemical properties. The protein content of KBPC, PBPC and control yogurt at 2.5 % fortification level were 6.08%, 5.87%, and 4.44% respectively. The pH of KBPC and PBPC fortified yogurt varies (pinto > kidney) from 4.57-4.12 whereas control yogurt samples vary from 4.61-4.19 during 28 days of storage. There were no significant differences obtained in color of 0 and 2.5% fortification level on day 1 for both bean types. The WHC of PBPC yogurt found to be higher as compared to the KBPC and control yogurt samples. The WHC of both bean type fortified yogurt increased with increase in the fortification level and decreased with respect to a storage period of 28 days. No significant difference has been observed in the means values of viable count between different bean types. Both the control and fortified yogurt samples have followed a shear thinning behavior ($n<1$). The consistency index of fortified yogurt samples was increased significantly ($p<0.05$) with the increase in the fortification level whereas the flow behavior index values decreased significantly with increase in fortification level.

The obtained results showed that the kidney and pinto beans protein could be used as potential source of providing new alternative fermented dairy product with

high protein content, no significant change in microbial properties but may affects the rheological properties of yogurt. However, on comparing PBPC and KBPC fortified yogurt, KBPC fortified yogurt was found better on the basis of protein content, total solids content, color, consistency and flow behavior index.

Chapter 1

Introduction and Background

1.1. Introduction

Food plays an important role in supplying adequate nutrients to fulfill the metabolic activity required by the human body whereas at the same time it must satisfy the feeling and well-being demanded by consumers (Homayouni 2008). Beyond meeting nutrition needs, food may play detrimental or beneficial roles in some diseases. Increasing awareness of the relationship between diet and health leads to generate food which encourages improving health, well-being state and reducing the risk of the disease. These foods are known as "Functional food." Functional foods are analog to the conventional foods that help to lower the risk of chronic disease and provides physiological benefit beyond its nutritional values (Homayouni, 2008; Camara et al. 2013).

Worldwide, yogurt is considered a most popular fermented dairy product due to its health and nutritional benefits (Tamime 2004; Chandan 2006). Yogurt was produced by the milk fermentation at the controlled condition with the help of *Lactobacillus bulgaricus and Streptococcus thermophilus* popular known as the thermophilic lactic acid bacteria. Henceforth, yogurt is recognized as an acidified coagulated functional dairy product (Bhattarai et al., 2016; Yu et al., 2016). Recently yogurt is gaining popularity due to its probiotic content. Fortification of yogurt with beans can be considered as a functional food that includes probiotic, prebiotic and symbiotic (Ziemer et al., 1998).

According to the definition given by WHO (World Health Organization), probiotics are “live microorganism added as supplement in the food to provide the health

benefits which influences the host animal especially by balancing its intestinal microbial activity" (Champagne and Gardener, 2005). "Prebiotics defined as a food ingredient that is non-digestible and acts as a substrate which promotes the growth of probiotics by inducing the activity of selective bacteria in the gastrointestinal tract.". "Symbiotic can be defined as a combination of prebiotics and probiotics that serve the purpose of both and provide the beneficial effect in promoting health by simulating the activity live beneficial microorganism." (Aswal et al., 2012).

Beans are seeds comprised amongst crop 'Pulse or Legumes'. These are stapled and traditional foods, especially among the developing countries. There are several kinds of beans include pinto beans, navy beans, black beans, kidney beans, etc. produced in the United States. Pinto beans (*Phaseolus Vulgaris L.*), is the most commonly consumed dry beans in the United States. USA is the top worldwide producer and distributor of pinto beans (USDA 2012). Despite these attributes, consumption of beans in the diet is substantially low. Dry beans contain many essential nutrients and very low in saturated fats. Dry beans are considered as a high source of protein and dietary fiber along with prebiotic and many diverse micronutrient compositions (Geil et al., 1994).

Dry beans constitute of different types of prebiotic including raffinose, stachyose, fructo-oligosaccharides and resistance starch (RS). All compounds act as substrates for beneficial bacteria that promote the microbial activity in the gastrointestinal tract (GI) and gut metabolism. The nutrient and phytochemical content of dry beans protect against many diseases such as cardiovascular disease, inflammation, oxidative stress, a

different type of cancer, and diabetes. It also helps in lowering the level of both HDL and LDL cholesterol (Camara et al., 2013).

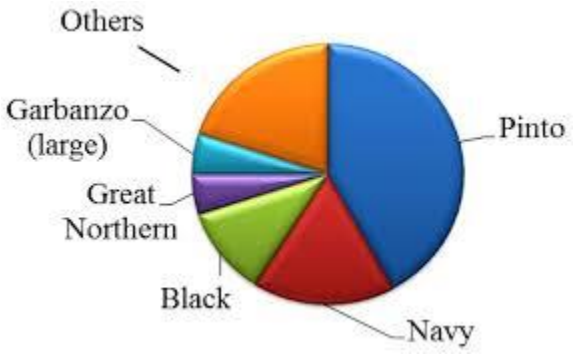


Fig. 1.1 Production of different types of beans in the United States (expressed in percent) USDA data. (Others comprised small white, light, and red kidney beans, black-eyed, lima, pink and cranberry).

High-protein diets with low-fat have become an appealing diet plan for the increasing health-conscious population. Legumes are an excellent source of protein with multifaceted applications in the food industry. Extracting proteins from beans and fortifying with yogurt provides new opportunities for increasing the quality and shelf life of the product. Therefore, prebiotic found in beans should be a good source of nutrients for probiotic in yogurt. There is an excellent potential to develop fortified yogurt as a new developed product. Besides the health benefits of yogurt, its physical properties, appearance, and texture properties are important for consumer acceptability (Ozen et al., 2009).

1.2. Hypothesis: Extraction of protein from both beans (kidney and pinto) and addition to yogurt may enhance the physio-chemical, microbiological, and rheological

characteristics of yogurt and can be offered as an alternative new fermented dairy product.

1.3. Objectives

- To extract protein from the selected beans flour and add them to the yogurt.
- To optimize the amount of final raw material flour that can be added to the yogurt.
- To analyze the Physio-chemical, microbiological and rheological behavior of fortified yogurt for 28 days shelf life studies.

The primary objectives of this thesis are analyzed in the following chapters:

Chapter 2: Study of Properties of Low-Fat Yogurt Fortified with Beans Protein Concentrate, and

Chapter 3: Study of Properties of Low-Fat Yogurt Fortified with Kidney Bean Protein Concentrate and its Comparison with Pinto Bean Protein Concentrate Fortified Yogurt

1.4. Review of Literature

1.4.1. Milk Fermentation

Milk fermentation processes were historically based upon spontaneous souring of milk caused by inherent microflora. Milk fermentation according to the modern process is under controlled, predicted and exacting condition, the result of which is yield cultured dairy products of good nutritional and sanitary standards (Chandan, 2006).

Milk fermentation can be defined as any modification of chemical or physical properties of milk or dairy products resulting from the fermentative action of a microorganism or their associated enzymes (Frank and Marth, 1998). When fermentation

of milk is talked, lactic acid bacteria (LAB) are the basis of every fermented milk product. Fermented or acidified milk are the preparations made from pasteurized milk, sometimes enriched with non-fat dry matter and flavoring, and acidified by lactic acid bacteria resulting in typical texture and flavor. The method of fermenting is an easy, economical and safe way of preserving milk. Lactic acid bacteria (LAB) change the conditions in the milk such that most of the undesirable organisms, including pathogens, cannot produce or die. A common characteristic of all acidified milk products is the presence of lactic acid produced by fermentation of lactose by various combination of thermophilic and mesophilic lactic acid bacteria leading to the coagulation of milk protein (casein) (Acharya, 2010).

Depending upon the fermentation type, fermented milk can be classified as lactic, mold-lactic as e.g. Villi, yeast-lactic as e.g. Kefir, acidophilus-yeast milk, and Koumiss. Depending on the characteristics of lactic microflora, lactic fermentation products are further classified, as mesophilic as e.g. Nordic ropy milk, Maziwalala, thermophilic as e.g. Labneh, Shrikhand, Skyr, Yogurt, Bulgarian buttermilk and probiotic therapeuticas e.g. Bio-fermented milks, acidophilus milk, AByogurt, Yakult, Danone, Cultura-AB (Fernandes, 2008).

1.4.2. Yogurt Background

Beyond the nutritional and health benefits of yogurt, itis considered as the most popular fermented dairy products worldwide with extensive acceptance for centuries (Weerathilake et al., 2014). Yogurt was consumed from long back many centuries with no accurate record of first made. According to legend, yogurt was developed as a preserved milk product by the ancient Turkish people in Asia. “Yoghurt” is originated

from Turkish verb “jugurt” that defined any fermented food with an acidic taste (Younus et al., 2002).

Yogurt’s shows a similar nutritional profile as milk components but may affect the more beneficial when supplemented with fruits, cereals, legumes or other components in term of health and nutritional values. Yogurt is an outstanding source of nutritive protein, vitamins which include B1, B2, and B12, minerals include calcium, phosphorus, zinc, magnesium, niacin, and folate. Yogurt considered as a good carrier for providing the minerals, vitamins, protein required for the human body with probiotics. Consumption of yogurt, a dairy product, increases the chances of meeting the daily required along with improves the overall diet plan and health system of human body. nutritional recommendation. The nutrition provided by the yogurt is of high biological value and bioavailability (Mckinley, 2005).

According to FDA (Food & Drug Administration, United States, 1996), yogurt can be produced by incorporating dairy ingredients with a bacterial culture mainly contains lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus*) that produces lactic acid. It can be developed with single or multiple dairy ingredients which include Skim milk, partial- skim milk, milk, cream, non-fat dry milk, whey, lactose, lactalbumins, and lactoglobulins to improve non-fat solids.

According to USDA (2001) specifications “Yogurt shall possess a clean, acid flavor and be free from undesirable flavors such as: bitter, rancid, oxidized, stale, yeasty and unclean” and also specifies that yogurt “shall possess a firm custard-like body with a smooth homogenous texture.” It was recommended production of yogurt should follow certain requirements based on the type of yogurt. Yogurt prepared from full-fat milk

should contain milk fat (3.5%), solid not fat (8.25%) with titratable acidity (0.9% as lactic acid). Yogurt prepared from low-fat milk and non-fat/skim milk should contain milk fat (0.5-2% and < 0.5%) (Sharma, 2011). Yogurt contains food must be homogenized, pasteurized or ultra-pasteurized before the addition of yogurt culture to ensure the destroy of microorganism. Codex Specification regarding live microorganisms is that at least 106 colony forming units (CFU) per gram must be present on the package. (Desai, 2012).

1.4.3. Yogurt Production

In the history of yogurt, it was developed inadvertently by natural process. Later its manufacturing process was vastly advanced. Some guidelines for yogurt manufacturing have been described below:

1.4.3.1. Milk standardization

Based on the guidelines and consumer demands, the fat content of yogurt should be in the range of 0.1% - 10% (Tamine and Robinson, 2007). For obtaining the desired level, fat and solid not fat content of milk should be standardized and term denotes as ‘milk standardization’. For achieving desired solid not fat content and improved firmness of yogurt, milk is fortifying with skim-milk or full-fat milk powder, casein powder or whey protein concentrate. This helps the manufacturer in obtaining the total solids content of yogurt which should be 9% for skim milk yogurt and more than 20% for other types of yogurt. (Walstra et al., 2006).

1.4.3.2. Homogenization

Milk homogenization refers to the breaking of the fat molecule from 2–10 μm to 0.1–1 μm in milk. Homogenization is done after pasteurization to prevent the formation

of a cream layer on the top of the product during yogurt manufacture process (incubation). This treatment is more significant for the manufacture of full-fat (Shah, 2006). Homogenization increases the surface area of milk fat globule membrane that interacts with protein networks (denatured whey and casein) and helps in gel formation (Cho et al., 1999). Homogenization also results in obtaining the good quality product by homogenizing the added materials, thus lowering the syneresis effect and affect the consumer by improving the firmness and whiteness of final product with good mouthfeel. (Amatayakul, 2005).

1.4.3.3. Heat treatment

Milk is heated before the addition of yogurt starter culture and process commonly known as pasteurization. This treatment helps in improving the physical properties, microstructure and inactivate the undesired microorganism which affects the yogurt starter culture with both temperature and time being critical factors. The heat treatment time-temperatures are generally in the range of 80-85 °C for 30 minutes or 90-95°C for 5 minutes and important for texture development. This combination denatures 70 to 80 percent of whey proteins. (Lucey, 2002 and 2004). It also forms some growth stimulating substances like cysteine, glutathione or thioglycolate for yogurt starter bacteria (Tamime and Robinson, 2007).

1.4.3.4. Inoculation and Incubation (Fermentation)

Inoculation and incubation is the important step in yogurt manufacture. After heat treatment milk brings down to cool at around 45°C and yogurt starter culture is added to

the milk and incubated at 40-44 °C. This is the optimum temperature ranges that help in the growth of *S. thermophilus and L. delbrueckiissp. bulgaricus* (Lee and Lucey, 2010). The significance involves in the fermentation is the production of lactic acid from lactose. During fermentation, pH of milk reduces to 4.6 or less. This reduces in pH results in milk protein coagulation and production of volatile compounds that are responsible for flavor and aroma of yogurt product (Weerathilake et al., 2014).

1.4.3.5. Cooling

Cooling is the final and significant step in yogurt production. After incubation, recommendedto cool the yogurt product at refrigerated condition (popularly around 5 °C). This step is significant in the starter culture metabolic activity of final product, as below 10 °C, the growth of enzyme was limited. Hence, the shelf life of the product is more when stored at a temperature below 10 °C. Cooling the yogurt at refrigerated condition also helps in controlling the acidity of the final product (Lucey, 2004).

1.4.4. Yogurt and Probiotics

The term “probiotic” was first used in 1954 that derives from Latin word pro meaning “for” and Greek word bios meaning “life” to stipulateconstituents that were essential for a healthy life. Another definition was provided by Parker (1974), which states “Probiotics as organisms and substances which contribute to intestinal microbial balance.” There are numbers of the definition given for probiotics but most scientifically accepted and the widely used definition given by FAO/WHO panel (FAO/WHO, 2001) which states probiotics as “Live micro-organisms that deliberate health benefit to host when provided in an adequate amount.” 'Probiotic' is a mono or mixed culture of live microorganisms and play a therapeutic role by lowering cholesterol, improving lactose

tolerance, modulating immunity, and preventing several cancers (Kailasapathy and Chin, 2000).

The most common species showing probiotic properties were from the *Lactobacillus* and *Bifidobacterium* genera. These microorganisms cause no harm to the human body as they exist and generally regarded as safe (GRAS). These bacteria are key microorganism responsible for the fermentation of milk and preservation of food products from the beginning of mankind. Raw milk and fermented dairy products such as fermented milk, yogurt, and cheese contain *Lactobacilli* are abundant in the diet and are found in the gastrointestinal tract soon after birth. The most used species of probiotics in foods for human consumptions are *Lactobacillus* and *Bifidobacterium* that promotes the significant health benefits associated with ingestion of beneficial microorganism. (Stamatova and Meurman, 2009).

For serving the food applications, the requirements of probiotics are to survive until it reaches to gastrointestinal tract where they apply their intended effect. They need to compete with the resident microbiota and must maintain its viability and metabolic activity in the intestinal ecosystem (Araujo et al., 2012).

1.4.5. Yogurt Classification

Yogurt is classified into diverse categories. It can be named as a standard cultured yogurt which is made with *L. bulgaricus and S. thermophilus*. These standard culture yogurt helps to promote the activity of microflora already present in the gut to maintain the intestinal health. On the other hand, bio yogurts are milder and less acidic, manufactured by culturing beneficial microorganisms that claim to have numerous health

benefits once ingested, typically the probiotic strains *of Bifidobacteria and L. acidophilus*. (Dowden, 2013).

The yogurt was further classified on the basis of product available in the market with different varieties of flavor, forms, and textures. Also, it can be classified according to the nature of yogurt, added flavor and incubation process.

1) Classified on the basis of chemical composition

It was mainly classified according to the fat content of yogurt. The three main type of yogurt available in the market were regular, low-fat and non-fat yogurt. The regular/plain yogurt atleast contain 3.25% milk fat and produced from whole milk. The fat content recommended for low-fat and non-fat yogurt were 0.5-2.0% and less and equal to 0.5% milk fat and produced from low-fat, partially skim milk or skim milk respectively (FDA, 2013).

2) Classified on the basis of Physical nature

According to this classification, yogurt is classified as solid, semi-solid and fluid. Set Yogurts are solid in nature (jelly-like texture). The acidification and coagulation of the processed milk for the set yogurt takes place in the consumer packaging. In set yogurt, until it consumed, the coagulum should not be broken hence the firmness of gel is an important factor. Overall, set yogurt should be, smooth in texture, firm, free from lump or graininess and spoon-able without any syneresis on the surface of the product. The set yogurt can be supplemented and produced as natural, fruit or flavored. On the other hand, stirred yogurt which is in the semi-solid and fluid state also known as fluid/drinking yogurt. Yogurts are produced by incubating the mix in a tank followed by breaking the coagulum by stirring gently before cooling and packaging are called stirred

yogurt. Drinking yogurt can be categorized as stirred yogurt with low viscosity. In drinking yogurt, the agitation used to "break" the coagulum is severe (Aswal et al., 2012; Weerathilake et al., 2014).

3) Classified on the basis of flavor

According to this classification, yogurt is further characterized according to the particular flavor into plain/ natural, fruit or flavored yogurt. Flavor addition produced a variety of yogurt with different taste that enhances the consumer appeal. Addition of flavor can be done before or after homogenization. The yogurt with no fortification/supplementation is called plain or natural yogurt, sometimes known as unsweetened fermented milk product. Its nutritional composition values are similar to milk from which yogurt is developed. Yogurts that are available in a nenormous collection of flavors including legumes, cereal, fruits and vegetables, chocolate, vanilla, etc. known as flavored yogurt. Generally, the addition of flavor was done during production of yogurt which results in different types of flavor (Weerathilake et al., 2014).

1.4.6. Fortification of yogurt and storage studies

Ozturkoglu-Budak et al. (2016) observed the effect of dried fruits (walnut, hazelnut, almond, or pistachio) for the fortification of yogurts. It was found fortified yogurts shows higher protein and total solid contents and lower syneresis compared with control yogurt on day 21. The microbiological viable counts of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were estimated for 21 days storage period. and found that addition of nuts, except walnut, results in the increased *S. thermophilus* and *L. bulgaricus* counts.

Santillán-Urquiza et al. (2017) described the effect of fortification of yogurt with micro and nano-minerals which include zinc, iron, and calcium on the properties of yogurt during a storage period of 28 days. From the study it was obtained, during the storage, there is a decrease in pH while the increase in acidity for all the samples. There was a net change in color recorded for the fortified yogurt during the storage. The yogurt shows the Non-Newtonian behavior with an increase in the consistency and firmness of the zinc and calcium nanoparticles fortified yogurt as compared to other samples. There is an increase in syneresis in the samples fortified with micro-mineral, while been lower in nano-fortified samples. There was no significant change found in yield stress and flow index during storage.

Staffalo et al. (2003) studied the rheological and sensory analysis of fiber-fortified yogurt. It was found from the studied that there was no significant difference in syneresis and pH of the fiber-fortified yogurt as compared to the control sample. The color of all fortified product except yogurt fortified with apple fiber shows no significant difference in comparison with control. The fortified product was accepted by the untrained panel during sensory evaluation of product although the addition of fiber to yogurt modified certain rheological properties as compared to control sample.

Singh et al (2007) explained the rheological analysis of fruit yogurt fortified with calcium. It was shown in the study that supplementation of calcium with fruit yogurt showed less thinning behavior as compared to control fruit yogurt. There was less decrease in the initial apparent viscosity found in fortified yogurt. The δ-values shows a similar bond nature and gel formation in both calcium fortified and controlled fruit yogurt. The water holding capacity (WHC) of fortified yogurt increased during the

storage of 14 days as compared to the control fruit yogurt. There was no significant difference found in the flavor, texture, and color of calcium-fortified yogurt with a comparison to control in the sensory analysis.

1.4.7. Application of pulse as an ingredient for the fortification of yogurt

Pulses are dry leguminous plant seeds that comprise of beans, peas, lentils, and chickpea. Pulses are well known for good source of protein. They are rich in nutrients includes carbohydrates, proteins, minerals, and vitamins, serves as a recommended essential human diet requirement. The consumption of pulses is gaining popularity in recent times due to the awareness of health benefits associated with this food group. Despite this awareness, pulses are still not being used. In order to increase utilization, and consumption of this product, different applications are considered. Pulses act as the substrate and assist the growth of probiotic and starter yogurt culture as growth nutrients for probiotic and yogurt starter cultures. Supplementation of milk with pulses provides alternative new product results in increased nutritional and health benefits of probiotic beverages such as yogurt.

Zare et al. (2011) studied the yogurt fortification with pulse and effect by yogurt and probiotic culture on acidification rate. Two different yogurt starter and probiotic were observed. Pea protein, chickpea flour, soy flour, soy protein concentrates, and pea fiber used as a fortification component. It was found that all the ingredients used for the fortification result in the improved acidification rate of probiotics culture with maximum rate shown by lentil and soy flour. The growth of Lactobacilli was found higher in fortified yogurt as compared to control. There was no negative impact found on

acidification rate with the addition of pulse in milk. There was enhanced acid production by lentil and pea flour.

Another study by Zare et al. (2011) observed that yogurt fortified with 1-3% lentil flour appeared to result in a higher acid production and an improvement on physical and rheological properties. However, the microbial population was the same in both supplemented samples and control samples. Another study indicated that *Lactobacillus debrueckii ssp bulgaricus* strongly benefited by the lentil supplement (Zare, Champagne, Simpson, Orsat, &Boye, 2012).

Ita et al. (2016) studied the fortification of navy bean extract on the quality of yogurt. Extract obtained by water extraction procedure and was added to 2% reduced fat milk inoculated with yogurt culture (Danisco YO-MIX 883 LYO 500 DCU), and was fermented for 4 – 8 hours at 42 ºC. The results showed there was a decrease in pH and increase in acidity during the storage of 21 days. There was no significant effect on the viscosity of yogurt with the addition of Navy bean extract. There was an increase in the total count but that was not significant over time.

From the study of Agil et al. (2013), results showed an enhancement of selective probiotic bacteria at the initial day and maintained overall microbial counts during a 28-day storage period with the addition of green lentil flour. Yogurt fortified with chickpea water extract had higher counts of *S. thermophiles* than that of the control one (Bakr, 2013).

Chen et al. (2016) studied the supplementation of 1%- 5% (w/v) chickpea flour with 2% low-fat milk to developed new fortified yogurt product. From the study,the physiochemical and microbiological analysis was performed during the storage of 21

days. Color and viscosity of fortified product show no significant difference as compared to control sample. There was a significant increase in the microbial growth (*Streptococcus thermophiles and L. delbrueckiisubsp*) of the fortified product during storage. Sensory analysis of control yogurt and fortified yogurt (1% and 2% chickpea flour) shows a similar score.

Chapter 2

Study of Properties of Low-Fat Yogurt Fortified with Pinto bean protein

Abstract

In recent years, popularity and consumption of yogurt have significantly increased. Such a trend is more because of the presence of probiotic culture which helps in maintaining good health and in combating intestinal disorders. Simultaneously, high-protein diets low in fat has become an appealing diet plan for the growing healthy conscious population. Legumes are an excellent source of protein with multifaceted applications in the food industry. Pinto bean is a most popular bean in the United States and northwestern Mexico. Our objective is to study the physio-chemical, microbiological and rheological properties of low-fat yogurt fortified with pinto bean protein concentrate (PBPC). Protein was extracted from the pinto beans (PB) by following the isoelectric point precipitation method. Protein concentrate was added to the yogurt at different fortification levels of 0, 2.5, 5, 7.5, and 10% before the inoculation stage. Control yogurt had 0% protein concentrate. The prepared yogurt samples were stored at 5^{o}C and analyzed. The yogurt samples were evaluated for their proximate composition (moisture, fat, protein, ash and total solid content), pH, titratable acidity, water holding capacity, color, microbiological, and rheological properties. PBPC-fortified yogurt samples had a significant influence on the protein content, WHC, viscosity and microbiological properties as compared to the control yogurt sample. Significant ($P<0.05$) increased in the protein content with an increase in the fortification level of protein concentrate. No significant difference was found in the means value of pH and TA for different days of

samples, whereas, for WHC, day 1 and days 7 are found to have significantly different means. No significant effect has been observed on microbial properties due to fortification, days and interaction of fortification level and days. The viscosity value increased during the 28-day period of storae in control and fortified samples

2.1. Introduction

Yogurt is fermented milk product (Zare et al. 2011) produced by the action of selected thermophilic lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These organisms are used as yogurt cultures to produce a characteristic mild clean lactic flavor and typical aroma (Alkali et al., 2007). Yogurt mainly recognized due to its nutritional benefits and positive reaction on the gut health (Tamime 2004 and Chandan 2006). The popularity of yogurt is increasing around all over the world mainly because of the beneficial action provided by bacteria present in the yogurt culture (Eroglu et al. 2014). In United states, a wide variety of yogurt (fruit yogurt, yogurt shake drinkable yogurt, yogurt ice-cream, yogurt mousse, fortified yogurt products etc.) are available in the market (Staffolo et al. 2003)

There were various researches on the fortification of yogurt with legumes such as lentil, chickpea, soybean, navy bean (Zare et al. 2011; Kucukcetin et al. 2012; Drake et al. 2000; Ita et al. 2015; Chen et al. 2016). These researches reported the effect of fortification of legumes powder at different concentration on the physical, chemical, rheological and microbiological characteristics of yogurt at certain storage conditions. It was found that beans are the good source for the fortification of dairy products as it improves the physical, microbial, and rheological properties of yogurt product (Zare et

al., 2011; Chen et al., 2016). Besides, beans are a good source of protein and composed of several types of prebiotic including resistance starch (RS) and the fructooligosaccharides, stachyose, and raffinose. These compounds serve as substrates for bacterial fermentation in the human intestine, thereby influencing the microbial ecology of the gastrointestinal (GI) tract and gut metabolism.

Pinto beans (*Phaseolus Vulgaris L.*) most common consumed dry beans in the United States. The United States is the top producer and distributor of pinto beans. Despite these attributes consumption of beans in diet is substantially low. Pinto beans contain many essentials nutrients such as protein, dietary fiber, folic acid, phosphorous, manganese, and very low in saturated fats. It is a good source of protein and dietary fiber along with prebiotic and many diverse micronutrient compositions. The nutrient and phytochemical content of dry beans protects against many diseases such as oxidative stress, inflammation, cardiovascular disease, diabetes, and many types of cancer. It also helps in lowering the level of both HDL and LDL cholesterol. Henceforth prebiotic found in beans should be good source of nutrients for probiotic in yogurt.

To develop a low-fat yogurt with desired gel network, it is recommended to add protein to yogurt. The strength of gel network is influenced by the fat content, decrease in fat content results in fragile gel network structure of yogurt and leads to less desirable rheological properties of yogurt that also influences the taste and flavor. Besides the nutritional quality of yogurt, it is important to develop a desired quality final yogurt product with physical and rheological characteristics. The increase in the protein content of yogurt strengthen the gel network as denaturated protein acts as a filler and binders within casein matrix.

Therefore, extracting protein from beans and incorporating it into yogurt to improve the overall quality will increase the consumption of beans as an alternative fortified product.

So far, no work has been done on protein extraction from pinto bean and supplementation to yogurt. A hypothesis was assumed that fortification of yogurt with extracted protein may enhance the overall quality of yogurt. The main objective of this research is to study the effect of yogurt fortification with pinto bean extracted protein on physio-chemical (moisture, fat, protein, ash, total solids content, pH, titratable acidity, water holding capacity, and color), microbiological and rheological characteristics of yogurt.

2.2. Materials and Methods

2.2.1. Raw Materials

Pinto beans were purchased from Walmart, Brookings, SD, USA. For preparing low fat yogurt, fresh 2% low fat milk was obtained from Dairy Plant, South Dakota State University, Brookings, SD, USA.

2.2.2. Sample Preparation and Protein Extraction

The protein from the pinto bean was extracted by the isoelectric point method. The beans were grinded into fine powder after the removal of bran. The raw material was partially grinded and dehulled to remove the bran. The slurry was prepared (15% dry solids) and mixed in high-speed mixer for 1h so that the particles get uniformly mixed. The mixed slurry was Centrifuged at 8000 rpm for 20 mins in an Accuspin^{TM} 400 Centrifuge. The supernatant obtained contains protein and pH was adjusted by adding

0.1N HCl solution. As at the isoelectric point, a protein has no net charge. After adjusting pH to isoelectric point, the solution was kept in a water bath at 60ºC for 30 minutes. The solution was centrifuged at 8000 rpm for 20 mins. Supernatant and sediment were separated. The sediment obtained contains a protein. The extracted protein was kept in a vacuum drying at 45ºC for 24-36 h. The dried protein was grinded into a fine powder by using Coffee Grinder. For better extraction efficiency of the protein, steps were repeated 2-3 times by making a slurry of obtained sediment after the first centrifuge (Fan et al., 1974).

2.2.3. Yogurt Preparation

Non-fat dry milk was added to the milk to increase the total milk solids to 14% before heating to 85°C for 30 min in a water bath. For yogurt preparation DVS yogurt culture (CHR Hansen Inc.) was used. It contains the active strains of *Streptococcus thermophiles, Lactobacillus delbruekii subsp. bulgaricus.* For inoculation, 125g of frozen pellet was thawed in the sterile container in the water bath at 25°C. The first dilution is made by adding the 10 g of defrosted DVS culture to 90g of cold milk. The sample was then fortified with the extracted protein and incubated at 42±1 °C until the pH reached to 4.6. It approximately took 4 h. The yogurt samples were immediately stored at 2-5 °C for further analysis. (Singh et al. 2007)

2.2.4. Physio-Chemical Analysis

2.2.4.1. Proximate Analysis

The moisture, fat, protein, ash, and totals solids (TS) contents of control and fortified yogurt was determined according to standard AOAC method (AOAC 2012).

2.2.4.2. pH and Titratable Acidity (TA)

The pH of yogurt samples was measured by using digital pH meter (pHTestr 30). For TA, 10 g of yogurt sample was taken in a flask and 30 ml of water was added for dilution. 1 ml of phenolphthalein indicator was added and titrated against standard NaOH solution to obtain the end point indicated by faint pink color (AOAC 2012). The titratable acidity indicates the freshness of yogurt

$$Titrable\ Acidity\ as\ Lactic\ Acid = \frac{9AN}{W} \quad \ldots(2.1)$$

Where,

A = Volume of standard NaOH required for titration

N = Normality of NaOH solution

W = Weight of sample taken

2.2.4.3. Water Holding Capacity (WHC)

The water Holding capacity (WHC) of control and fortified yogurt was measured by centrifugation method. Twenty grams of yogurt (Y) was centrifuged for 10 min at 669g and 20 ºC. The whey expelled (WE) was removed and weighed. (Singh et al. 2007) The WHC is expressed in % was defined as

$$WHC\ (\%) = \frac{100 * (NY - WE)}{NY} \quad \ldots(2.2)$$

2.2.4.4. Color

The color values (L*, a*, and b*) of the yogurt samples were measured using a Minolta Spectrophotometer (Model CM-2500d, Minolta Corporation, Ramsey, NJ).

2.2.5. Microbiological Analysis

Total Viable Bacterial Count was measured by plate count agar medium. The sample (0.1ml) of each dilution was taken onto each sterile Petri dish and poured plate count agar medium. The plates were then incubated at 37◦C for 24 hours. The total bacterial count was measured in colony forming unit per gram (cfu/ml). (Hasan et al., 2016; Olabisi et al. 2017).

2.2.6. Rheological Analysis

Yogurt gels were stirred by manual rotating them very slowly (2-3 s each rotation) 10 times with a Tablespoon inside a cup. The yogurt samples appear visually homogenous. The following test was performed with viscoanalyzer rheometer using a plate and plate geometry with 2 mm gap setting and at 25 ºC constant temperature. Flow curve characteristics was performed at shear rates between 30 s^{-1} and 200 s^{-1}. Both delay time and integration will study at 1 s. The data obtained was adjusted to the power law equation:

$$\text{Shear stress} = K*(\text{Shear rate})^{n} \quad \ldots(2.3)$$

Where K is the consistency index, and n is the power law index. The values of n explain the flow behavior of curve as Newtonian (n is close to 1) or non- Newtonian (n is far from 1). The apparent viscosity curved was studied with respect to shear rate during a storage period of 28 days.

2.2.7. Statistical Analysis

All statistical analysis was performed using the ANOVA test for multiple sample comparison to test for any significant differences in the mean values of all groups (SAS

9.1, SAS Institute Inc. NC, US). Tukey's test was performed for the comparison of samples. A p-value ≤ 0.05 was regarded as statistically significant.

2.3. Results and Discussion

2.3.1. Physio-chemical Properties

The physio-chemical properties include proximate analysis (moisture content, protein content, fat content, ash content, total solids content), pH, titratable acidity (TA), water holding capacity (WHC), and color.

2.3.1.1. Effect of PBPC fortification on Proximate Analysis

Table 2.1 represents the ANOVA model procedure for all the physio-chemical property of the fortified yogurt with pinto bean extracted protein at different fortification level. The moisture content of fortified yogurt was lower than that of control yogurt. The moisture content of control yogurt (0%) obtained on day 1 is 87.5 % and 88% on day 28. The obtained moisture values were slightly higher than the moisture content reported by Karam et al. (2013) for a yogurt fortified with milk protein at a different concentration which was 80-85%. The Fig. 2.1(a) represents the moisture content values of the fortified and control yogurt at different fortification level during storage. Table 2.2 represents the interaction of fortification effect on physiochemical properties based on Tukey's test. Table 2.3 and Table 2.4 represents the interaction of Days effect on physiochemical properties based on Tukey's test.

The protein content obtained for the fortified yogurt was higher than the control sample (0%). The protein content increased with increase in fortification level. There was 33%, 71%, 101%, 129% increase in percentage protein content at fortification level of

0%, 2.5%, 5%, 7.5% and 10% respectively as compared to control sample (0%) on day1. The reference for protein intake in the diet is 0.8g per kg body weight. The Fig. 2.1(b) represents the protein content values of the fortified and control yogurt at different fortification level during storage.

No increase was there in fat content during the storage period, but significant increase was found in fat content with increase in fortification levels. The fat content of control yogurt was 1.96g/100g whereas fat content (g/100g) in fortified yogurt at different fortification level (2.5-10%) were 2.23, 2.37, 2.54, 2.94, respectively.

There found an increase in ash content from day 1 to day 28 for both control and fortified yogurt sample. The ash content of fortified yogurt was higher than control yogurt for storage period of 28 days. Fig. 2.1(c) represents the ash content values of the fortified and control yogurt at different fortification level during storage.

The total solids content depends on the fortification level. An increase in total solids content was observed with an increase in fortification level. The highest value of total solids content obtained at 10% fortification level was 18.38 % on day 1 and 19.02% on day 28. Fig. 2.1(d) represents the total solids content values of the fortified and control yogurt at different fortification level during storage.

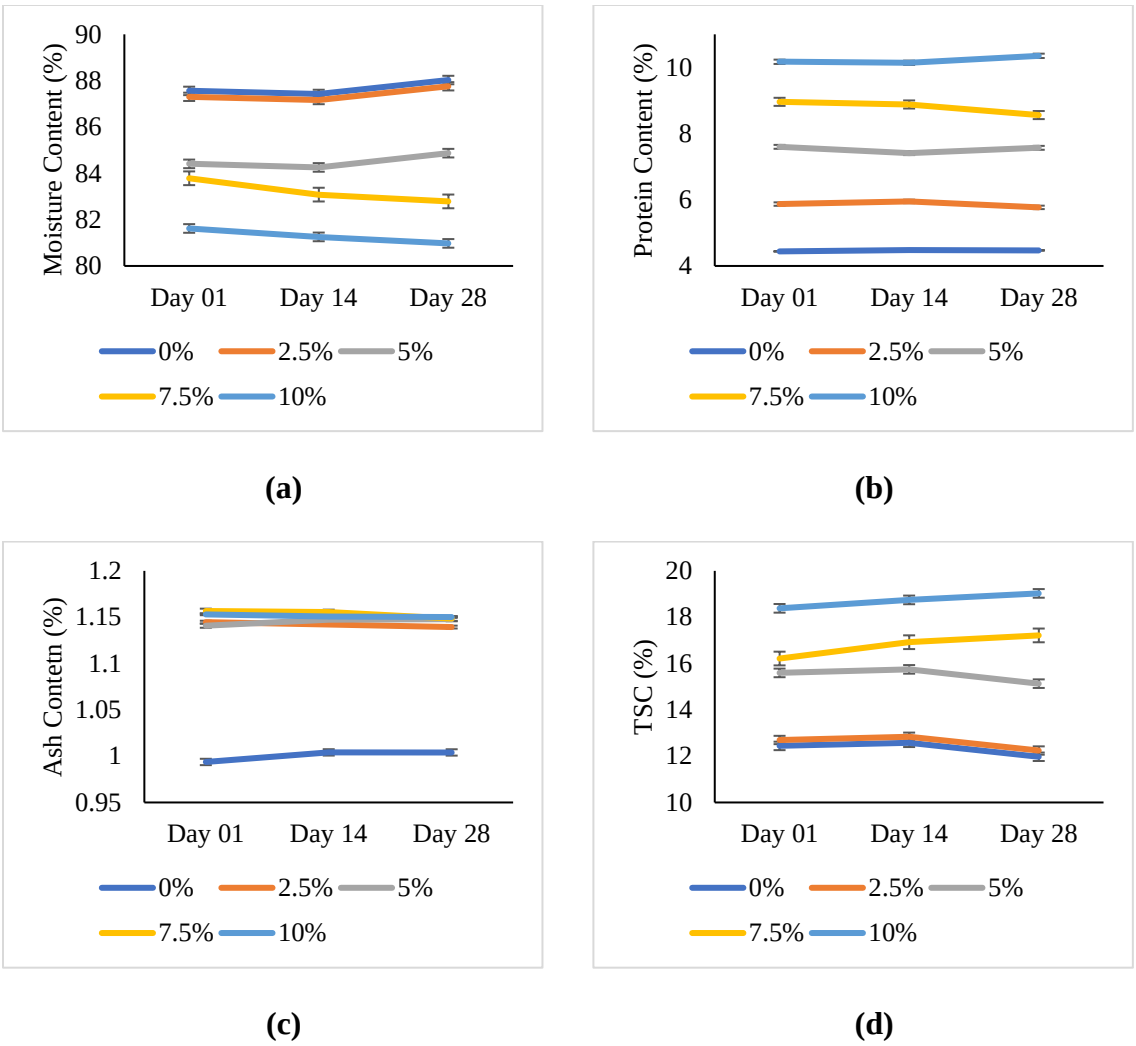


Fig. 2.1 Proximate analysis of the control (0%) and fortified yogurt samples with PBPC at different fortification level during storage of 28 days (a) Moisture Content (b) Protein content (c) Ash Content (d) Total Solids Content (TSC)

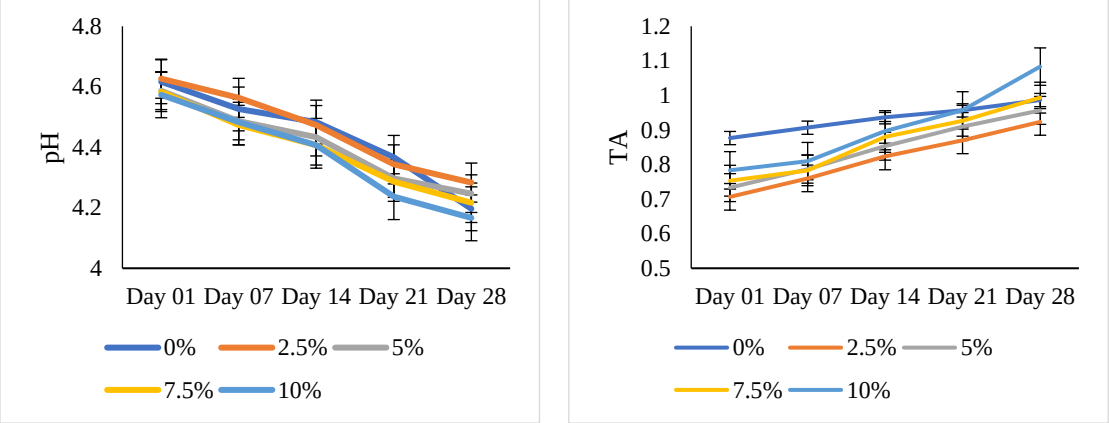
2.3.1.2. Effect of PBPC fortification on pH and Titratable Acidity (TA)

Both pH and TA, express the acidity of yogurt and play an important role in the flavor of yogurt. Maximum TA and minimum pH were found on the 28th day of the storage period. It is recommended that the pH of yogurt should be 4.6 or less and TA

should not be less than 0.7% as lactic acid. Chen (2016) found the similar results for pH and TA during the storage period of 21 days for the chickpea fortified yogurt.

The highest value of pH 4.62 was obtained at 2.5% fortification level on day 1 whereas the lowest value 4.16 was obtained at 10% fortified yogurt on day 28. The pH value of control yogurt on day 1 was 4.61, and on day 28 was found to be 4.19.

The highest value of TA (1.08%) as the lactic acid was obtained at 10% fortification level on day 28 whereas lowest value (0.70%) as the lactic acid was obtained at 2.5% fortified yogurt on day 1. The TA value of control yogurt on day 1 and day 28 were 0.87 and 0.98 % as lactic acid, respectively.



(a) **(b)**

Fig.2.2 Chemical analysis of the control (0%) and fortified yogurt samples with PBPC at different fortification level during storage of 28 days (a) pH (b) Titratable Acidity (TA)

2.3.1.3. Effect of PBPC fortification on Water Holding Capacity (WHC)

The water holding capacity of yogurt fortified with pinto bean protein flour was higher than that of the control sample. Similar results were reported by Ahmet et al.

(2012) & Zare et al. (2011) that yogurt with supplementation of lentil flour increased the water holding capacity with an increase in fortification level. The protein content plays an important role in yogurt as it helps in stronger texture and less whey separation (Peng et al. 2009).

The water holding capacity of control sample on day 1 was 86.87% which was found to be almost similar in day 7, then started decreasing till day 21 and stabilized afterward. The water activity of control sample on day 28 was 82.65%. The highest water activity was observed at 10% fortification level on day 1.

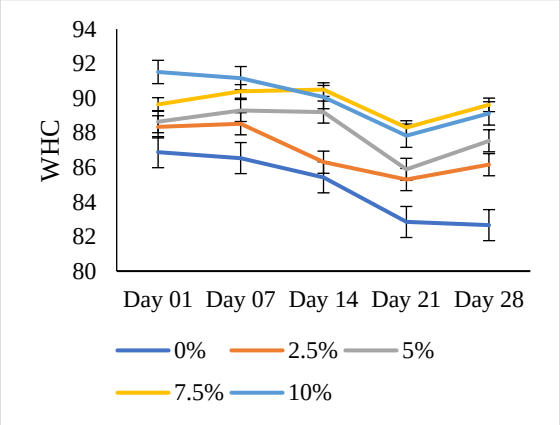


Fig. 2.3 Water holding capacity (WHC) of the control (0%) and fortified yogurt samples with PBPC at different fortification level during storage of 28 days

2.3.1.4. Effect of PBPC fortification on Color

Color is the important parameter for any food product in the market responsible for consumer acceptance. Even though functional products provide good health benefits but without good attractive product, consumer will not accept. The yogurt at 10% fortification level was good in protein content, but it was darkest in color than control and others fortified yogurt. The L* represents the lightness or darkness (0-100), a* represents

red or green hues (positive (+ve) or negative (-ve)) and b* represents yellow or blue hues (+ve or -ve) (Zare et al. 2011).

The L* of control yogurt on day 1 was 93.68 which explains the lightness of product or whiter in color, a* was -1.94 which explains the redness and b* was 8.25 which explains the yellow hues. The 2.5% fortified yogurt explains the same factor with little change in value of L*, a*, b*. The color of yogurt became darker with increase in fortification level and days. Similar results were reported by Zare et al. 2011. Fig. 2.4 represents color L*, a*, b* of the fortified and control (0%) yogurt at different fortification level during storage of 28 days.

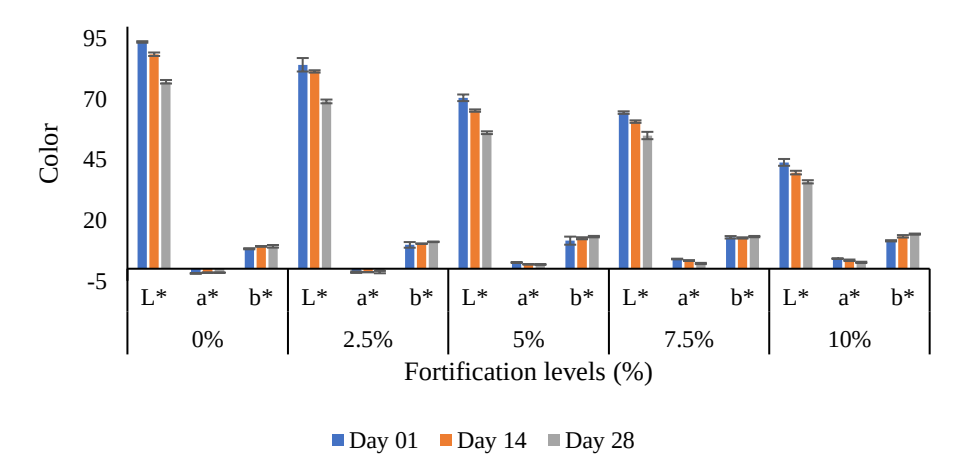


Fig. 2.4 Color (L*, a*, b*) of the control (0%) and fortified yogurt samples with PBPC at different fortification level during storage of 28 days

Table 2.1 ANOVA model procedure for all the physio-chemical property of the fortified yogurt with pinto bean extracted protein at different fortification level.

The ANOVA Model											
Fortification Comparison	**Ash**	**Fat**	**Moisture**	**Protein**	**Total Solid**	**pH**	**TA**	**WHC**	**Color *L**	**Color *a**	**Color *b**
Model	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
R-square	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.93
ANOVA for Main and Interaction Effect (p-values at the 0.05 level)											
F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
D	0.8304	-	0.0053	<.0001	0.0053	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
F * D	0.8903	-	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003

Note: F represents fortification and D for days. The p-values greater than 0.05 (red colored fonts) have a significant effect on respective physiochemical properties due to fortification level, bean types and number of days as main effect and their interaction. From above table, it is clear that ANOVA models for different physio-chemical properties are statistically significant as p-value is less than 0.05 and R^2 values are also quite high (above 0.92 for all the physio-chemical properties). No significant effect has been observed on the mean of fat, protein, pH, TA, WHC, color L*, a* and b* due to fortification level, different days and their interaction, however, significant effect has been observed due to either of two-way of fortification levels and days as well as days itself on ash content.

Table 2.2 Fortification Comparison for physiochemical properties based on Tukey's method

Comparisons non-significant at the 0.05 level are indicated by *.											
Fortification Comparison	**Ash Content**	**Fat Content**	**Moisture Content**	**Protein Content**	**Total Solid Content**	**pH**	**TA**	**WHC**	**Color *L**	**Color *a**	**Color *b**
10.0 - 7.5	*0.0	0.4	-1.9	1.4	*1.9	0.0	0.0	*0.2	-20.25	*0.2	*0.1
10.0 - 5.0	*0.0	0.6	-3.2	2.7	*3.2	0.0	0.1	1.8	-24.2	1.4	*0.6
10.0 - 2.5	*0.0	0.8	-6.1	4.4	*6.1	-0.1	0.1	3.0	-38.4	4.9	2.6
10.0 - 0.0	0.2	1.0	-6.4	5.8	*6.4	-0.1	0.0	5.1	-46.7	5.2	4.2
7.5 - 10.0	*0.0	-0.4	1.9	-1.4	*-1.9	0.0	0.0	*-0.2	20.3	*-0.2	*-0.1
7.5 - 5.0	*0.0	0.2	-1.3	1.3	*1.3	0.0	0.0	1.6	-3.9	1.1	*0.5
7.5 - 2.5	*0.0	0.4	-4.2	2.9	*4.2	0.0	0.1	2.8	-18.2	4.6	2.6
7.5 - 0.0	0.2	0.6	-4.5	4.3	*4.5	-0.1	-0.1	4.8	-26.4	4.9	4.1
5.0 - 10.0	*0.0	-0.6	3.2	-2.7	*-3.2	0.0	-0.1	-1.8	24.2	-1.4	*-0.6
5.0 - 7.5	*0.0	-0.2	1.3	-1.3	*-1.3	0.0	0.0	-1.6	3.9	-1.1	*-0.5
5.0 - 2.5	*0.0	0.2	-2.9	1.7	*2.9	0.0	0.0	1.2	-14.2	3.5	2.0
5.0 - 0.0	0.1	0.4	-3.2	3.1	*3.2	0.0	-0.1	3.2	-22.5	3.8	3.6
2.5 - 10.0	*0.0	-0.8	6.1	-4.4	*-6.1	0.1	-0.1	-3.0	38.4	-4.9	-2.6
2.5 - 7.5	*0.0	-0.4	4.2	-2.9	*-4.2	0.0	-0.1	-2.8	18.2	-4.6	-2.6
2.5 - 5.0	*0.0	-0.2	2.9	-1.7	*-2.9	0.0	0.0	-1.2	14.2	-3.5	-2.0
2.5 - 0.0	0.1	0.3	*-0.3	1.4	0.3	0.0	-0.1	2.1	-8.2	0.3	1.5
0.0 - 10.0	-0.2	-1.0	6.4	-5.8	*-6.4	0.1	0.0	-5.1	46.7	-5.2	-4.2
0.0 - 7.5	-0.2	-0.6	4.5	-4.3	*-4.5	0.1	0.1	-4.8	26.4	-4.9	-4.1
0.0 - 5.0	-0.1	-0.4	3.2	-3.1	*-3.2	0.0	0.1	-3.2	22.5	-3.8	-3.6
0.0 - 2.5	-0.1	-0.3	*0.3	-1.4	-0.3	0.0	0.1	-2.1	8.2	-0.3	-1.5

- Mean value of ash content from different samples are significantly different between 0% and all other fortification levels i.e., 5%, 7.5% and 10%.
- Mean value of fat, protein, pH, TA, and Color *L from different samples are significantly different among all the fortification level.
- Mean value of moisture content from different samples are significantly different among all the fortification level except between 0% and 2.5%
- No significant differences have been observed in TSC mean values among most of the fortification level except between 0% and 2.5%.
- Mean value of WHC from different samples are significantly different among all the fortification level except between 7.5% and 10%
- Mean value of Color *a from different samples are significantly different among all the fortification level except between 7.5% and 10%
- Mean value of Color *b from different samples are significantly different between 0% and all other fortification levels i.e., 5%, 7.5% and 10%.

Table 2.3 Days Comparison for physiochemical properties based on Tukey's test

Comparisons non-significant at the 0.05 level are indicated by *.							
Days Comparison	**Ash Content**	**Moisture Content**	**Protein Content**	**Total Solids Content**	**Color *L**	**Color *a**	**Color *b**
Day 14 - Day 28	*0.002	*-0.2	0.0	0.2	8.5	0.4	-0.6
Day 14 - Day1	*0.002	-0.3	0.0	0.3	-4.2	-0.4	0.8
Day 28 - Day 14	*-0.002	*0.2	0.0	-0.2	-8.5	-0.4	0.6
Day 28 - Day1	*0.000	-0.1	-0.1	*0.1	-12.7	-0.8	1.4
Day1 - Day 14	*-0.002	0.3	0.0	-0.3	4.2	0.4	-0.8
Day1 - Day 28	*0.000	0.1	0.1	*-0.1	12.7	0.8	-1.4

- No significant difference in means value of ash content is found for different days of samples.
- Except Day 14 and Day 28, all have significantly different means values of moisture content.
- Mean values of protein, and all the colors are significantly different among the different days samples.
- Except Day 1 and Day 28, all have significantly different means values of total solids content

Table 2.4 Days Comparison of pH, TA and WHC based on Tukey's test

Comparisons non-significant at the 0.05 level are indicated by *.			
Days Comparison	**pH**	**TA**	**WHC**
Day 7 - Day 1	-0.1	0.0	*0.2
Day 7 - Day 14	0.1	-0.1	0.9
Day 7 - Day 28	0.3	-0.2	2.2
Day 7 - Day 21	0.2	-0.1	3.1
Day 1 - Day 7	0.1	0.0	*-0.2
Day 1 - Day 14	0.2	-0.1	0.7
Day 1 - Day 28	0.4	-0.2	2.0
Day 1 - Day 21	0.3	-0.2	3.0
Day 14 - Day 7	-0.2	0.1	-0.9
Day 14 - Day 1	-0.1	0.1	-0.7
Day 14 - Day 28	0.2	-0.1	1.3
Day 14 - Day 21	0.1	0.0	2.3
Day 28 - Day 7	-0.3	0.2	-2.2
Day 28 - Day 1	-0.4	0.2	-2.0
Day 28 - Day 14	-0.2	0.1	-1.3
Day 28 - Day 21	-0.1	0.1	1.0
Day 21 - Day 7	-0.2	0.1	-3.1
Day 21 - Day 1	-0.3	0.2	-3.0
Day 21 - Day 14	-0.1	0.0	-2.3
Day 21 - Day 28	0.1	-0.1	-1.0

No significant difference in means value of pH and TA is found for different days of samples, whereas, in WHC, only day 1 and days 7 are found to have significantly different means.

2.3.2. Microbiological Analysis

From the Table 2.5, it is clear that the ANOVA model was found to be highly significant as p-value <0.05 and R^2 value equals 0.97. No significant effect has been observed on microbiological properties due to fortification, days and interaction of Fortification level and days.

Table 2.6 and 2.7 signifies comparison of differences in mean of total viable count using Tukey's Studentized Range of Fortification and Days, respectively. The mean

value of microbial property from different samples were not significantly different among all of the fortification level. However, significant difference has been found in microbial property values for all the days.

The total viable count obtained for control sample on day 1 was 2.11×10^8 cfu/ml. The total viable count obtained in fortified yogurt was higher than the control sample. This may be due to the prebiotic properties of beans and total solids content. Seleet et al. (2016) also found a similar increase in the number of total viable count of fortified yogurt than the control sample. Fig. 2.5 represents the total viable count of the fortified and control (0%) yogurt at different fortification level during storage of 28 days.

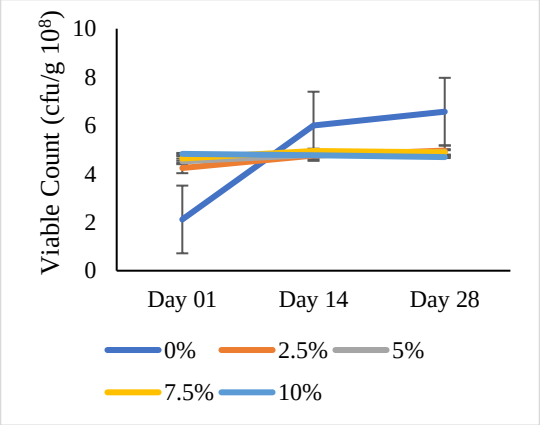


Fig. 2.5 Total viable count of the control (0%) and fortified yogurt samples with PBPC at different fortification level during storage of 28 days

Table 2.5 ANOVA Procedure for microbiological analysis

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	36.76	2.63	61.76	<.0001
Error	30	1.28	0.043		
Corrected Total	44	38.03			

R-Square	Coeff Var	Root MSE	Viable Mean
0.96	4.32	0.20	4.77

Table 2.6 Comparison of differences in mean of total viable count using Tukey's Studentized Range of Fortification

Comparisons significant at the 0.05 level are indicated by ***.			
Fortification Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**	
0 - 7.5	0.1	-0.2	0.4
0 - 10	0.1	-0.2	0.4
0 - 5	0.2	-0.1	0.4
0 - 2.5	0.2	0.0	0.5
7.5 - 0	-0.1	-0.4	0.2
7.5 - 10	0.1	-0.2	0.3
7.5 - 5	0.1	-0.2	0.4
7.5 - 2.5	0.2	-0.1	0.5
10 - 0	-0.1	-0.4	0.2
10 - 7.5	-0.1	-0.3	0.2
10 - 5	0.0	-0.3	0.3
10 - 2.5	0.1	-0.2	0.4
5 - 0	-0.2	-0.4	0.1
5 - 7.5	-0.1	-0.4	0.2
5 - 10	0.0	-0.3	0.3
5 - 2.5	0.1	-0.2	0.4
2.5 - 0	-0.2	-0.5	0.0
2.5 - 7.5	-0.2	-0.5	0.1
2.5 - 10	-0.1	-0.4	0.2
2.5 - 5	-0.1	-0.4	0.2

Table 2.7 Comparison of differences in mean of total viable count using Tukey's Studentized Range of Days

Comparisons significant at the 0.05 level are indicated by ***.				
Days Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Day 28 - Day 14	0.2	0.1	0.3	***
Day 28 - Day1	1.1	1.0	1.3	***
Day 14 - Day 28	-0.2	-0.3	-0.1	***
Day 14 - Day1	1.0	0.8	1.1	***
Day1 - Day 28	-1.1	-1.3	-1.0	***
Day1 - Day 14	-1.0	-1.1	-0.8	***

2.3.3. Rheological Properties

Rheological parameters of the yogurt samples described by the power law model are represent in the Table 2.8. Determination coefficient (R^2) for the model was above 0.91 showing satisfactory fit of flow curve. The consistency index increased significantly with the increase in the fortification level. Yogurt with 10% fortification showed a highest consistency index value as compare to the control and other fortification levels. Similar increase in consistency index value with increase in the fortification of lentil flour in yogurt has been observed by Kucukcetin et al. (2012).

The control and fortified yogurt showed a pseudo-plastic behavior as flow behavior index (n) value of $n<1$. The flow behavior index has been decreased significantly with increase in the fortification level. The values of flow behavior index varied from 0.43-0.34. The consistency and flow behavior index decreased with increase in the storage period. Similarly, Singh et al. (2008) reported the decrease in the value of the consistency and flow behavior index of calcium fortified yogurt during the storage studies of 14 days.

Table 2.8 Rheological parameters of stirred yogurt samples.

Storage time (Days)	1			7			14			21			28		
Pinto bean protein	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**
0% (Control)	10.1	0.42	0.96	9.32	0.41	0.97	8.67	0.4	0.96	8.32	0.39	0.96	8.22	0.39	0.97
2.5%	11.14	0.43	0.99	10.44	0.42	0.96	9.85	0.41	0.95	9.6	0.4	0.95	9.51	0.38	0.99
5.0%	13.57	0.41	0.99	12.9	0.4	0.99	12.36	0.39	0.98	12.11	0.37	0.99	11.87	0.36	0.98
7.5%	15.47	0.39	0.99	14.82	0.38	0.98	14.28	0.37	0.99	13.93	0.34	0.95	13.75	0.33	0.92
10%	16.98	0.38	0.98	16.09	0.37	0.96	15.51	0.36	0.99	15.14	0.35	0.94	14.9	0.34	0.94

Note – K represents Consistency index ($Pa.s^n$), n represents Flow behavior index (Dimensionless), R^2 represents the Determination Coefficient.

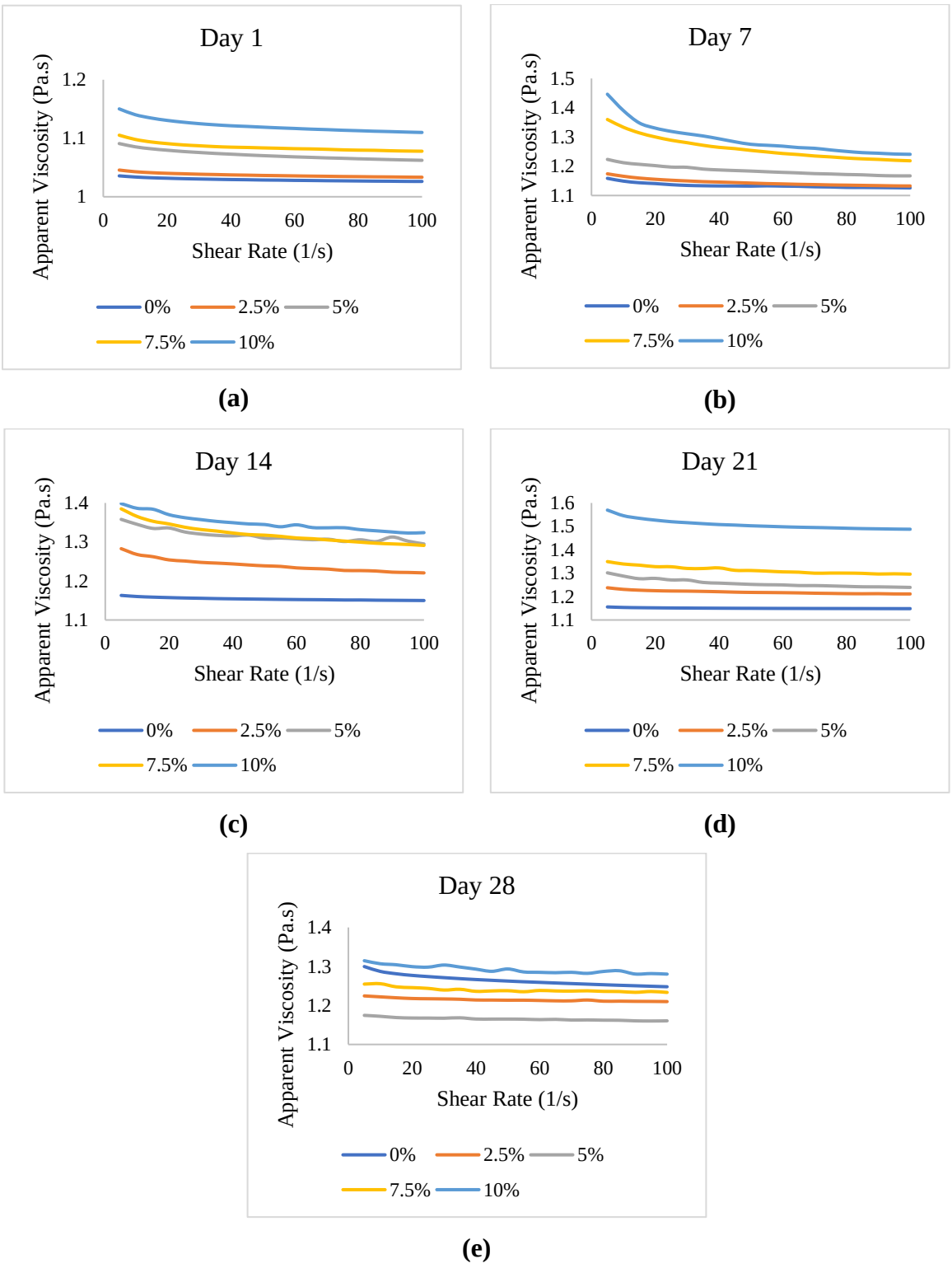


Fig. 2.6 Apparent viscosity vs shear rate curve for control (0%) and PBPC fortified yogurt during storage of 28 days (a) Day 1 (b) Day 7 (c) Day 14 (d) Day 21 (e) Day 28

The apparent viscosity with respect to time for all the fortification level during storage of 28 days were shown in Fig. 2.6. The apparent viscosity was found to be increasing with increasing fortification level and storage period. The apparent viscosity was found to be decreasing with increasing time that represents the shear-thinning behavior of yogurt. No significant difference was observed in the apparent viscosity of the control yogurt and fortified yogurt on day 1 but there was sudden increase in the apparent viscosity of fortified yogurt on day 7 as compared to control. Results shows that there was increase in viscosity with fortification, but no significant difference was obtained between groups.

2.4. Conclusions

The study showed that the physio-chemical and nutritional properties of yogurt are influenced by the fortification of the pinto bean protein concentrate. All the physiochemical properties for pinto beans protein fortified yogurt were found to be statistically significant with p-value less than 0.05. The pH and TA value indicated that the addition of pinto bean protein flour significantly enhanced the acidification of yogurt. The lowest pH value and the highest TA value were recorded on 28^{th} day of storage with 10% fortification level. PBPC fortification of yogurt results in increase in WHC of yogurt. No significant effect has been observed on microbial properties due to fortification, days and interaction of fortification level and days. The viscosity value of both control and fortified yogurt increased during 28-day period of storage and expressed a shear-thinning behavior.

Therefore, pinto bean protein has the potential of providing more nutritional benefits and high quality in fermented dairy products on the basis of physio-chemical, microbial, color, viscosity evaluation. It can be utilized as a natural supplement to fortify yogurt.

Chapter 3

Study of Properties of Low-Fat Yogurt Fortified with Kidney Bean Protein Concentrate and its Comparison with Pinto Bean Protein Concentrate Fortified Yogurt

Abstract

The effect of different legumes (Kidney and Pinto) protein fortification on the physiochemical, microbiological and rheological properties of yogurt were evaluated in this chapter. The kidney bean protein was extracted by isoelectric point precipitation method and added to the yogurt before inoculation at different fortification level (0%, 2.5%, 5%, 7.5% and 10%) where 0% represents the control yogurt sample. On comparing results, it was found from ANOVA that PBPC and KBPC fortified yogurt samples were statistically significant with p-value less than 0.05 and R^2 values to be more than 0.93 for all the physiochemical properties. Based on the Tukey's comparison according to the bean type fortification on the physiochemical properties it was found yogurt fortified with KBPC is high in protein content, total solids content and titratable acidity. The pH value of KBPC fortified yogurt is lower with a difference of 0.02. The color of KBPC is lighter as compared to PBPC but the water holding capacity of PBPC fortified yogurt is more with a difference of 2.07. Based on bean type no significant differences were obtained on the microbiological properties. Both PBPC and KBPC fortified yogurt shows increase in apparent viscosity with storage signifies a shear-thinning behavior. However, no significant differences were obtained in rheological properties due to the different beans types.

3.1. Introduction

Increase in consumer demand for healthy and nutritious food around the world, leads to the innovation and development of functional food products, by means of fortification or enrichment (Ozturkoglu-Budak et al., 2016). According to World Health Organization (WHO), and Food and Agricultural Organization (FAO) of the United Nation, 'Fortification' is defined as a supplementation or addition of essential nutrient to food more than naturally found, to improve its nutritional quality and public health benefits (Swieca et al., 2014). Nestle (2013) mentioned that natural resources are the best source for the fortification of food products to improve the overall quality with minimal risk effect.

To match the consumer expectation, there is a need to evaluate and modify the product continuously. Declaration of the year 2016 as pulse year by the UN has opened up avenues, raised awareness of health benefits and encouraged the consumption of beans, peas, chickpeas, and lentils. McCrory et al. (2010) and Ooi & Liong, (2010) indicates the positive effect of pulse consumption that attributes to the pulse properties which includes the presence of resistance fiber that helps in reducing the risk of cardiovascular disease, osteoporosis and gastrointestinal disorder. Pulses also have a low glycemic index as such it does not cause immediate spikes in blood sugar levels (Leterme, 2002) when consumed, making them great for people living with diabetes. It has also encouraged the use of pulses in new product development and research. As a result, ideas on how to use the different varieties of beans in creating new products, enhancing or substituting old products is ongoing. During the past few years there has been an intense focus on the emergence of plant-based proteins used in product development. This has given birth to the idea of extraction of protein and fortification.

Yogurt is the most popular nutritious fermented dairy product among entirely age groups all over the world (Tamime 2004; Chandan 2006; Erogule et al., 2016). Popularity of yogurt in the United States has been increased since last two decades due to diet pattern, age, protein consumption and diversified products range available in the market such as yogurt shake, fruit yogurt, drinkable yogurt, yogurt ice-cream, yogurt mousse, fortified yogurt products etc. (Fiszman & Salvador,1999; Staffolo et al., 2004). Yogurt is a cultured dairy product and produced by milk fermentation by lactic acid bacteria mainly *Lactobacillus delbruekiisupsp. Bulgaricus and Streptococcus thermophilus* that makes complex gel due to denaturation of milk protein and milk fat globules (Yu et al., 2016; Loveday et al., 2013).

The final strength of the gel network structure is directly influenced by the fat content of yogurt (Xu et al., 2008). The decrease in fat content results in a fragile gel network structure of yogurt and leads to less desirable rheological properties of yogurt that also influences the taste and flavor (Lobato-Calleros et al., 2014). Besides the nutritional quality of yogurt, it is essential to develop an acceptable final yogurt product. To develop a low-fat yogurt with desired gel network, it is recommended to add protein to yogurt. The increase in the protein content of yogurt offers an alternative way to strengthens the gel network. (Yu et al., 2016).

Both Kidney and pinto beans are dried beans and very rich in nutrient composition and a great source of protein. Despite the highly dense nutritional property of beans, their consumption in the diet is substantially low. McCrory et al. (2010) state the reasons behind the low consumption of this nutrient dense food may include food preference and taste, a cook time of pulses, insufficient education about the nutritional

benefits of these seeds, and most importantly the gastrointestinal disturbance that may result from high consumption of pulses. Therefore, it is better to utilize abundant protein source from bean to enhance the yogurt quality.

Several authors studied the fortification of yogurt with dried fruits, dried dairy ingredients, whey protein, milk protein, calcium fiber, wheat flour, cereals and legumes and its effects on the physiochemical and rheological characteristics of yogurt (Ozturkogl-Budak et al. 2016; Zare et al. 2011; Seleet et al. 2016; Kucukcetin et al. 2012; Drake et al. 2000; Staffolo et al., 2003). Yogurt mainly recognized due to its healthy value and nutritional benefits such as antagonistic, antimutagenic, anticarcinogenic, reduction of serum cholesterol. It is the excellent source of probiotic because of the presence of a large number of live probiotic bacteria. Recently research shows that yogurt is beneficial for protein enhancement for people following vegan diet and sportsmen, also improving lactose digestion for individuals with lactose maldigestion (Zare et al., 2011). Yogurt also contains mainly bioavailable proteins, vitamins, and minerals.

Therefore, the main objective of this chapter is to study the fortification of yogurt with kidney bean protein concentrate and comparing the physio-chemical, microbiological, and rheological characteristics of yogurt fortification with extracted protein from pinto and kidney beans at different fortification levels.

3.2. Materials and Methods

3.2.1. Raw Materials

Kidney beans were purchased from the Walmart and 2% low-fat milk was obtained from Dairy Plant, South Dakota State University, Brookings, SD, USA.

3.2.2. Sample Preparation and Protein Extraction

Kidney beans were converted into fine flour particle using Perton Lab Mill. For obtaining the uniform particle size, the beans flour was sieved using size 500µm. For extracting the protein from kidney beans according to isoelectric point method, the slurry was prepared by adding water so that the dry solids should be 15% and mixed in high-speed mixer for 1h so that the particles get uniformly mixed. The mixed slurry was Centrifuged at 8000 rpm for 20 mins in Accuspin^{TM}400 Centrifuge. Supernatant and sediment were separated and collected in a beaker. Supernatant contains protein and the pH of the supernatant was measured using Digital pH meter (pHTestr 30). To adjust the pH of the supernatant according to the isoelectric point of the beans 0.1N HCl was added. As at the isoelectric point, a protein has no net charge, the protein gets separated after heating solution in a water bath at 60ºC for 30 minutes. The solution was then centrifuged at 8000 rpm for 20 mins and sediment obtained contains protein. The extracted protein was kept in a vacuum drying at 45ºC for 24-36hrs. The dried protein was ground into a fine powder by using Coffee Grinder. After that proximate analysis of the protein, extract was determined. For more recovery of the protein, steps were repeated 2-3 times by making a slurry of obtained sediment after the first centrifuge. (Fan et al. 1974)

3.2.3. Milk Base Preparation

Milk was standardized according to the required factors for the yogurt such as total solids is maintained by addition of skim milk powder. Non-fat dry milk (NFDM) was added to milk to increase total milk solids to 14% before heating to 85 ºC for 30 minutes and then cooled to 42-44° C spontaneously (Singh et al. 2007).

3.2.4. Yogurt Preparation

3.2.4.1. Inoculation of Culture

Frozen yogurt culture pellets were obtained DVS yogurt culture (CHR Hansen Inc.) It contains the active strains of *Streptococcus thermophiles, Lactobacillus delbruekii subsp. Bulgaricus*. For inoculation, 125g of frozen pellet was thawed in the sterile container in the water bath at 25°C. The first dilution was made by adding the 10 g of defrosted DVS culture to 90g of cold milk. (Singh et al. 2007)

3.2.4.2. Addition of Extracted Protein Powder

Protein extracted from kidney and pinto beans were added to the milk at the rate of 0%, 2.5%, 5%, 7.5% and 10% fortification level respectively. 0% fortification represents the control yogurt sample.

3.2.4.3. Fermentation/incubation

After addition of culture in respective milk base, it was mixed thoroughly with a respective sterile stirrer. The samples were incubated at 42±1 °C until the pH reaches to 4.6. It generally takes 4 hrs. The yogurt samples were immediately stored at 2-5 °C for further analysis. (Singh et al. 2007; Kucukcetin et al. 2012)

3.2.5. Analysis of Yogurt

Yogurt samples were brought to room temperature (preferably 25°C), and then the sample was mixed carefully by means of a spatula which passes from lower layer to the surface layer of the sample to displace and mix them well.

3.2.5.1. Proximate Analysis

The proximate analysis (moisture, fat, protein, ash and total solids content was determined according to the standards AOAC methods (2012)

3.2.5.2. pH and Titratable Acidity (TA)

Digital pH meter (pHTestr 30) was used to measure the pH or active acidity of control and fortified yogurt samples. Titratable Acidity (% as lactic acid) was measured by titrating homogenously mixed yogurt samples (10g) with 0.1 N NaOH using phenolphthalein as an indicator as per AOAC (2012) method.

3.2.5.3. Water Holding Capacity

About 20g of native yogurt (NY) sample was centrifuged for 10 min at 669 × g and 20 ºC. The whey expelled (WE) was removed and weighed. (Singh et al. 2007) The WHC is expressed in % was defined as:

$$WHC\ (\%) = \frac{100 * (NY - WE)}{NY} \quad \text{...(3.1)}$$

3.2.5.4. Color

The color values (L*, a*, and b*) of the yogurt samples were measured using a Minolta Spectrophotometer (Model CM-2500d, Minolta Corporation, Ramsey, NJ). The measure of lightness L* (0 to100) represents the black to white, a* (-100 to 100) represents green to red and b* (-100 to 100) represents blue to yellow (Zare et al. 2011)

3.2.5.5. Microbiological Analysis

The laboratory work area and the containers of the yogurt were swabbed thoroughly with 70% ethanol before starting the sample preparation for total plate count, to avoid contamination. The yogurt samples were shaken vigorously to suspend microbial content at room temperature. Sterilized test tubes were used for preparing the dilution. 1ml of the yogurt sample was transferred into a sterilized test tube and diluted serially in one-tenth stepwise to 10-10 dilution factor. Each (1ml) of the dilution 10^{-5} and 10-8 were plated into the Nutrient Agar culture in triplicate using the pour plate method. The plates

were then incubated at 37 ºC for 24 hours. After incubation, the colonies on nutrient agar plates were counted and used to determine the Total Viable Bacterial count of the yogurt samples expressed as CFU/ml (Olabisi et al. 2017).

3.2.5.6. Rheological Characteristics

Yogurt gels were stirred by manual rotating them very slowly (2-3 s each rotation) 10 times with a Tablespoon inside a cup. The yogurt samples were appeared visually homogenous. The following test was performed with viscoanalyzer rheometer using a plate and plate geometry with 2 mm gap setting and at 25 ºC constant temperature. Flow curve was performed at shear rates between 30 s^{-1} and 200 s^{-1}. Both delay time and integration will study at 1 s. The data obtained will adjust to the power law equation:

$$\text{Shear stress} = K*(\text{Shear rate})^n \quad ...(3.2)$$

Where K is the consistency index and n is the power law index. The values of n explain the flow behavior of curve as Newtonian (n is close to 1) or non- Newtonian (n is far from 1).

3.2.5.7. Statistical Analysis

All statistical analysis was performed using ANOVA test for multiple sample comparison to test for any significant differences in the mean values of all groups (SAS 9.1, SAS Institute Inc. NC, US). Data reported with standard errors. Tukey's test was performed for the comparison of samples. A p-value ≤ 0.05 was regarded as statistically significant.

3.3. Results and Discussion

3.3.1. Physio-chemical Properties

The physio-chemical properties include proximate analysis (moisture content, protein content, fat content, ash content, total solids content), pH, titratable acidity (TA), water holding capacity (WHC), and color. From the Table 3.1, the ANOVA model was found statistically significant for all the physiochemical properties as p-value < 0.05

3.3.1.1. Effect of KBPC fortification on Proximate Analysis

The mean value of moisture content from different samples are significantly different among all the fortification level. The moisture content of control yogurt obtained was 87.5% whereas for fortified yogurt values lies from 79-87% for kidney bean. There was decreased in the moisture content with an increase in the fortification level respectively. No significant differences were found in moisture content values between the Days 1 and Days 28. The Fig. 3.1 (a) represents the mean values for the effect of fortification level on moisture content with 28 days of storage period.

The protein content of KBPC fortified yogurt is higher than the control yogurt and increased with increase in fortification level (2.5-10%). Mean values of protein content from different samples were significantly different among all the fortification level. The highest protein content obtained were 14.43g/100g for KBPC fortified yogurt whereas the protein content of control was 4.44%. Ozturkoglu-Budak et al. (2016) also reported the protein content of control yogurt sample was 4.4±0.05 (wt/wt%) on day 1 and 4.59±0.03 (wt/wt%) on day 28. The Fig. 3.1 (b) represents the mean value of the protein content of yogurt fortified with kidney bean extracted protein.

There was an increase in ash content from day 1 to day 28 for both control and fortified yogurt sample. The ash content of fortified yogurt is higher than control yogurt. The Fig. 3.1 (c) represents the ash content at different fortification level of kidney bean fortified yogurt during a storage period of 28 days. From the Table 3.3, no significant difference was found in ash content values for different days of samples. The Mean value of ash content from different samples are significantly different among all the fortification level except 7.5% and 10% fortification level.

The mean value of fat content from different samples are significantly different among all the fortification level. The fat content increases with increase in the fortification level but no changes were obtained with the storage period of 28 days.

The total solids content depends on the fortification level. There was an increased in total solids contents with an increase in fortification level. The highest value of total solids content obtained was 20.60 (%wt/wt). However, the mean value of total solids content from different samples are significantly different among all the fortification level. It was found that there is no significant difference between the total solids content values during storage. Ozturkoglu-Budak et al. (2016) reported the similar result that the TSC for fortified yogurt is higher than control and there is no significant difference in TSC during storage for yogurt fortified with dried nuts. The total solids content values were shown in the Fig. 3.1 (d) at different fortification during storage.

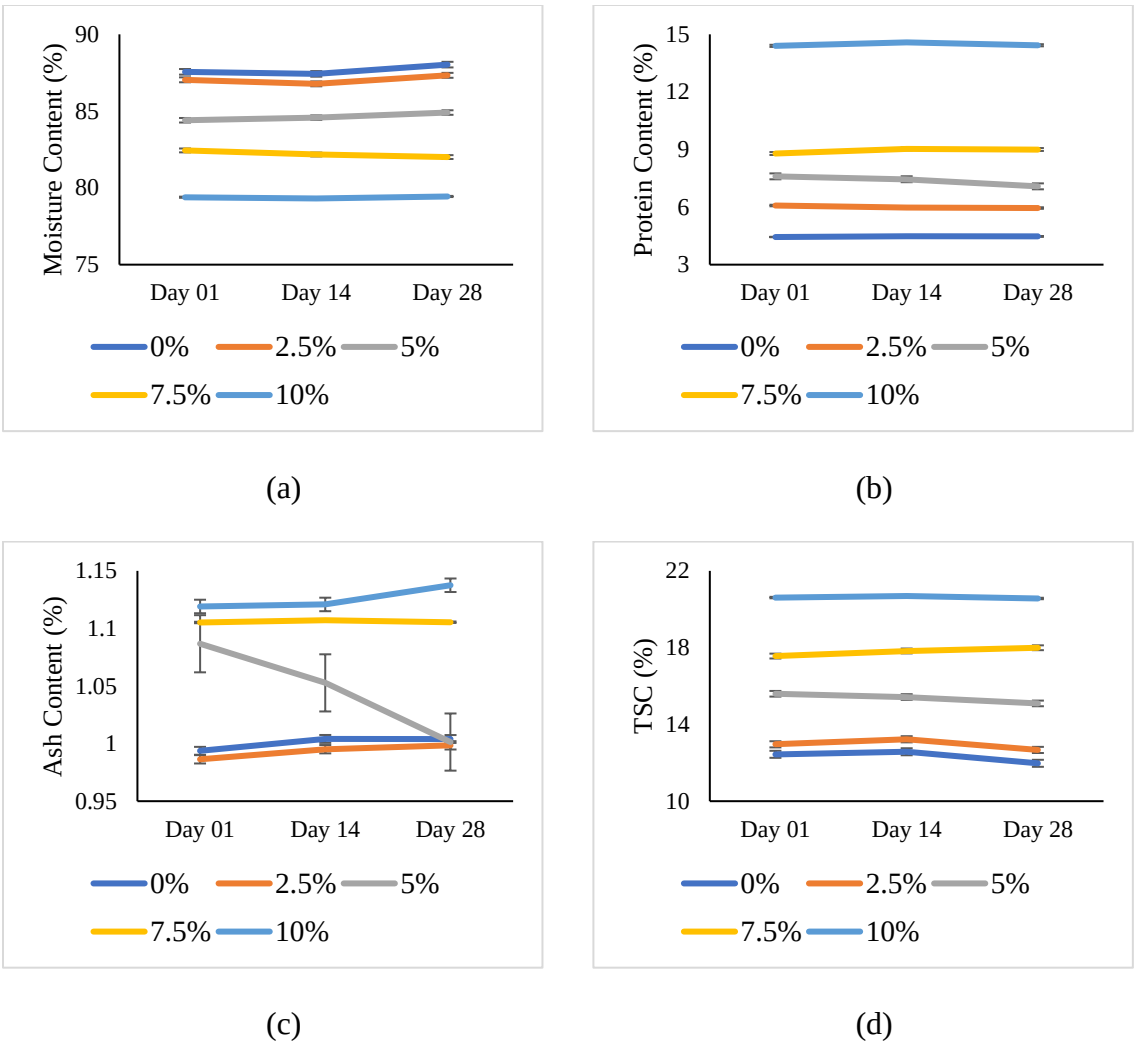


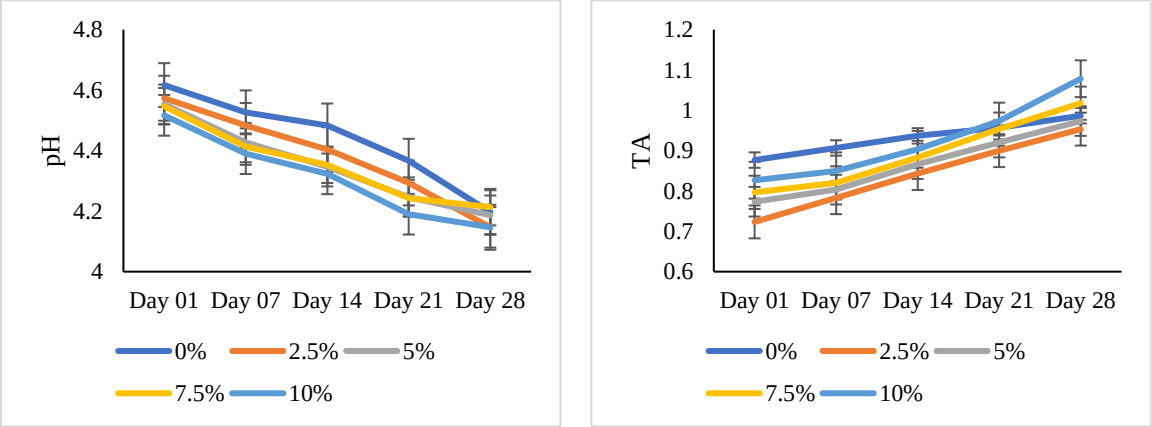
Fig. 3.1 Proximate analysis of the control (0%) and fortified yogurt samples with KBPC at different fortification level during storage of 28 days (a) Moisture Content (b) Protein Content (c) Ash Content (d) Total Solids Content (TSC)

3.3.1.2. Effect of KBPC fortification on pH and TA

It was observed from the study, the pH values of both control and KBPC fortified yogurt decreased with the storage periods of 28 days. The minimum pH indicates the acidity of yogurt appropriately. In accordance with FDA, the recommended value for pH of yogurt should be 4.6 or lower to express the acidity of yogurt. The pH of the control sample reached to 4.61 with 4hrs time duration whereas the pH values of fortified yogurt

were found to be less than 4.6 within 4hrs time duration. There was a slightly decreased in pH value of fortified yogurt with an increase in the fortification level. The minimum value of pH obtained was Chen et al. (2016) observed a similar decrease in pH with increase in fortification and storage periods. The Fig. 3.2 (a) represents the pH value of yogurt fortified with protein extract from kidney beans at different fortification level with respect to days.

Titratable acidity represents the acidification of yogurt and responsible for the taste and flavor of yogurt. According to FDA, TA of yogurt should not be lower than 0.7 % as expressed as lactic acid. TA of all samples increases with the number of days and with fortification level. The mean value of TA from different samples are significantly different among all the fortification level. Fig. 3.2 (b) represents the TA value of yogurt fortified with protein extract from kidney beans at different fortification level with respect to days.



(a) (b)

Fig. 3.2 Chemical analysis of the control (0%) and fortified yogurt samples with KBPC at different fortification level during storage of 28 days (a) pH (b) Titratable Acidity (TA)

3.3.1.3. Effect of KBPC fortification on Water Holding Capacity

The water holding capacity of fortified yogurt is higher than the control and water holding capacity increased with increase in the fortification level. Similar results were reported by Ahmet et al. (2012) & Zare et al. (2011) that yogurt with supplementation with lentil flour increases the water holding capacity with an increase in fortification level. Peng et al. (2009) studied that the fortified yogurt with increasing solids and protein content results in stronger texture and less whey separation. Water holding capacity represents the gel instability. The Fig. 3.3 represents the water holding capacity of KBPC fortified yogurt at different fortification level with respect to days.

From the ANOVA analysis, it was found WHC were significantly different among all the fortification level as p-value <0.05 and R^2 value equals 0.98. The highest water holding capacity obtained with kidney bean fortified yogurt with 10% fortification level was 90.45% on day 1. The Water holding capacity of control sample varies from 82-86% whereas kidney bean fortified yogurt samples varies from 81-90% respectively. The water holding capacity decreased with respect to days. However, no significant difference was found between Day 1 and Day 7 samples.

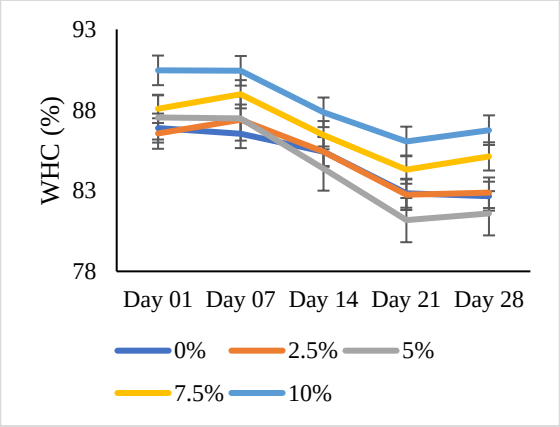


Fig. 3.3 Water Holding Analysis of the control (0%) and fortified yogurt samples with KBPC at different fortification level during storage of 28 days

3.3.1.4. Effect of KBPC fortification on Color

Color is the important parameter for any product in the market responsible for consumer acceptance. Even though functional products provide good health benefits, but without good attractive product consumer will not accept. Hence the yogurt 10% fortification level is good in protein content, but it is darker in color than control and fortified yogurt. The L* represents the lightness or darkness (0-100), a* represents red or green hues (+ive or -ive) and b* represents yellow or blue hues (+ive or -ive) (Zare et al. 2011). Fig. 3.7 represents the color value of yogurt fortified with protein extract from kidney beans at different fortification level with respect to days. The L* values decreased with increase in the fortification level that signifies the yogurt with high fortification level is darker in color. The a* values for control yogurt is negative which indicates the green hue whereas the fortified yogurt indicates the both green and red hue with respect to fortification levels.

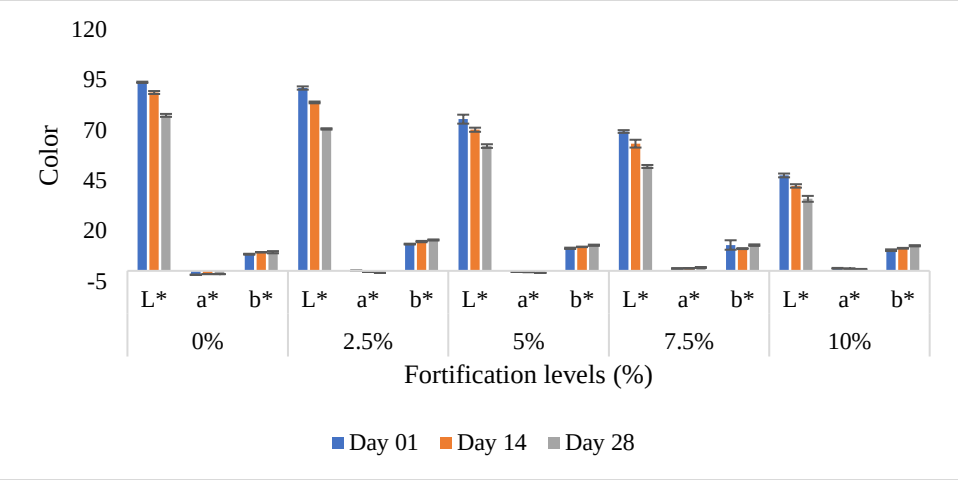


Fig. 3.4 Color (L*, a*, b*) of the control (0%) and fortified yogurt samples with KBPC at different fortification level during storage of 28 days

Table 3.1 ANOVA model procedure for all the physio-chemical properties of the fortified yogurt with extracted protein from pinto and kidney bean at different fortification level.

The ANOVA Model											
	Ash	**Fat**	**Moisture**	**Protein**	**Total Solids**	**pH**	**TA**	**WHC**	**Color L***	**Color a***	**Color b***
Model	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
R-square	0.97	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.93
ANOVA for Main and Interaction Effect (p-values at the 0.05 level)											
F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
B	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2010
F * B	<.0001	0.0022	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
D	0.4208	-	0.0006	<.0001	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
F* D	0.0049	-	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
B * D	0.4695	-	0.2271	<.0001	0.2271	<.0001	0.0003	<.0001	<.0001	<.0001	0.4544
F * B * D	0.0006	-	0.5008	<.0001	0.5008	<.0001	0.0041	<.0001	<.0001	<.0001	0.6268

Note: F represents fortification, B represents beans, and D for days. The p-values greater than 0.05 (red colored fonts), have significant effect on respective physiochemical properties due to fortification level, bean types and number of days as main effect and their interaction.

Table 3.2 Fortification Comparison for physio-chemical properties based on Tukey's method

Comparisons non-significant at the 0.05 level are indicated by *.											
Fortification Comparison	**Ash Content**	**Fat Content**	**Moisture Content**	**Protein Content**	**Total Solids Content**	**pH**	**TA**	**WHC**	**Color *L**	**Color *a**	**Color *b**
10.0 - 7.5	*0.0	0.3	-2.4	3.5	2.4	0.0	0.0	1.0	-19.9	*0.0	*-0.4
10.0- 5.0	0.0	0.6	-4.2	4.9	4.2	0.0	-0.1	2.9	-25.8	1.6	*0.0
10.0 - 2.5	0.1	0.7	-6.9	6.4	6.9	-0.1	-0.1	3.2	-39.2	3.2	*-0.2
10.0 - 0.0	0.1	0.9	-7.3	7.9	7.3	-0.1	-0.1	4.3	-45.7	4.0	3.3
7.5 - 10.0	*0.0	-0.3	2.4	-3.5	-2.4	0.0	0.0	-1.0	19.9	*0.0	*0.4
7.5 - 5.0	0.0	0.2	-1.9	1.4	1.9	0.0	0.0	1.9	-5.8	1.6	*0.4
7.5 - 2.5	0.1	0.4	-4.5	2.9	4.5	0.0	-0.1	2.2	-19.2	3.2	*0.2
7.5 - 0.0	0.1	0.6	-5.0	4.4	5.0	-0.1	-0.1	3.3	-25.7	4.0	3.7
5.0- 10.0	0.0	-0.6	4.2	-4.9	-4.2	0.0	0.1	-2.9	25.8	-1.6	*-0.4
5.0 - 7.5	0.0	-0.2	1.9	-1.4	-1.9	0.0	0.0	-1.9	5.8	-1.6	*0.0
5.0 - 2.5	0.0	0.2	-2.7	1.5	2.7	0.0	0.0	0.3	-13.4	1.6	*-0.2
5.0- 0.0	0.1	0.4	-3.1	3.0	3.1	-0.1	-0.1	1.4	-19.9	2.4	3.3
2.5 - 10.0	-0.1	-0.7	6.9	-6.4	-6.9	0.1	0.1	-3.2	39.2	-3.2	*0.2
2.5 - 7.5	-0.1	-0.4	4.5	-2.9	-4.5	0.0	0.1	-2.2	19.2	-3.2	*-0.2
2.5 - 5.0	0.0	-0.2	2.7	-1.5	-2.7	0.0	0.0	-0.3	13.4	-1.6	*0.2
2.5 - 0.0	0.1	0.2	-0.4	1.5	0.4	0.0	0.0	1.1	-6.5	0.8	3.5
0.0 - 10.0	-0.1	-0.9	7.3	-7.9	-7.3	0.1	0.1	-4.3	45.7	-4.0	-3.3
0.0 - 7.5	-0.1	-0.6	5.0	-4.4	-5.0	0.1	0.1	-3.3	25.7	-4.0	-3.7
0.0- 5.0	-0.1	-0.4	3.1	-3.0	-3.1	0.1	0.1	-1.4	19.9	-2.4	-3.3
0.0 - 2.5	-0.1	-0.2	0.4	-1.5	-0.4	0.0	0.0	-1.1	6.5	-0.8	-0.4

Note: Values marked with an asterisk (*) mark indicated the significant differences in respective fortification level.

- Mean value of ash content from different samples are significantly different among all the fortification level except 7.5% and 10% fortification level.
- Mean value of fat, protein, moisture, total solids, pH, TA, WHC, Color L* from different samples are significantly different among all the fortification level.
- Mean value of Color a* from different samples are significantly different among all the fortification level except between 7.5% and 10%
- Mean value of COLOR b* from different samples are significantly different between 0% and all other fortification levels i.e., 5%, 7.5% and 10%.

Table 3.3 Beans Comparison for physio-chemical properties based on Tukey's test

Comparisons non-significant at the 0.05 level are indicated by *.											
Beans Comparison	**Ash Content**	**Fat Content**	**Moisture Content**	**Protein Content**	**Total Solids Content**	**pH**	**TA**	**WHC**	**Color *L**	**Color *a**	**Color *b**
Kidney -Pinto	-0.06	-0.11	-0.63	0.88	0.63	-0.05	0.02	-2.07	2.35	-1.20	0.18
Pinto - Kidney	0.06	0.11	0.63	-0.88	-0.63	0.05	-0.02	2.07	-2.35	1.20	-0.18

Table 3.4 Days Comparison for physio-chemical properties based on Tukey's test

Comparisons non-significant at the 0.05 level are indicated by *.							
Days Comparison	**Ash Content**	**Moisture Content**	**Protein Content**	**Total Solid Content**	**Color *L**	**Color *a**	**Color *b**
Day 14 - Day 1	0.000*	-0.2	0.0	0.2	5.0	0.2	0.6
Day 14 - Day 28	0.004*	-0.3	0.1	0.3	14.3	0.5	-0.8
Day 1 - Day 14	0.000*	0.2	0.0	-0.2	-5.0	-0.2	-0.6
Day 1 - Day 28	0.004*	*-0.1	0.1	*0.1	9.3	0.3	-1.4
Day 28 - Day 14	-0.004*	0.3	-0.1	-0.3	-14.3	-0.5	1.4
Day 28 - Day 1	-0.004*	*0.1	-0.1	*-0.1	-9.3	-0.3	0.8

Table 3.5 Days Comparison for pH, TA and WHC based on Tukey's test

Comparisons non-significant at the 0.05 level are indicated by *.			
Days Comparison	**pH**	**TA**	**WHC**
Day 1 - Day 7	0.1	0.0	*-0.2
Day 1 - Day 14	0.2	-0.1	1.4
Day 1 - Day 21	0.3	-0.1	3.7
Day 1 - Day 28	0.4	-0.2	3.0
Day 7 - Day 1	-0.1	0.0	*0.2
Day 7 - Day 14	0.1	-0.1	1.6
Day 7 - Day 21	0.2	-0.1	3.9
Day 7 - Day 28	0.3	-0.2	3.3
Day 14 - Day 1	-0.2	0.1	-1.4
Day 14 - Day 7	-0.1	0.1	-1.6
Day 14 - Day 21	0.1	-0.1	2.4
Day 14 - Day 28	0.2	-0.1	1.7
Day 21 - Day 1	-0.3	0.1	-3.7
Day 21 - Day 7	-0.2	0.1	-3.9
Day 21 - Day 14	-0.1	0.1	-2.4
Day 21 - Day 28	0.1	-0.1	-0.7
Day 28 - Day 1	-0.4	0.2	-3.0
Day 28 - Day 7	-0.3	0.2	-3.3
Day 28 - Day 14	-0.2	0.1	-1.7
Day 28 - Day 21	-0.1	0.1	0.7

3.3.2. Microbiological Analysis

The total viable count of the control and fortified yogurt was obtained by the total plate count by incubating sample on nutrient agar. The total viable count obtained in fortified yogurt is higher than the control sample. This may be due to the prebiotic properties of beans and total solids content. Seleet et al. (2016) found a similar increase in a number of total viable count of fortified yogurt than the control sample. Mean value of the microbial property from different samples are not significantly different among most of the fortification level except 0% with 2.5% and 5%. However, a significant difference has been found in microbial property values for all the days. There was an increase in the total viable count with an increase in storage time. There was an increase in the total viable count of control yogurt as compared to fortified yogurt on the 28th day. Increase in total solids content results in an increase in a number of populations in fermented milk but the addition of protein content not increased at the similar rate as control sample increased during storage.

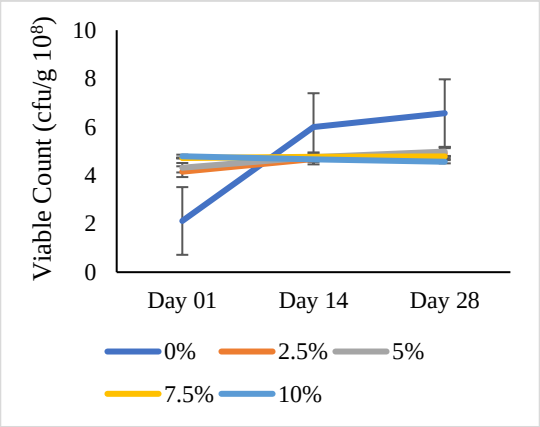


Fig. 3.5 Total Viable Count of the control (0%) and fortified yogurt samples with KBPC at different fortification level during storage of 28 days

Table 3.6 ANOVA model procedure for microbiological analysis of the fortified yogurt with extracted protein from pinto and kidney bean at different fortification level

ANOVA Model	
Model	**<.0001**
R-Square	**0.97**
Source	**Pr > F**
F	0.0013
B	0.1602
F*B	0.9598
D	<.0001
F*D	<.0001
B*D	0.9129
F*B*D	0.9280

Table 3.7 Fortification Comparison for microbiological properties based on Tukey's method

Comparisons significant at the 0.05 level are indicated by *.**				
Fortification Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
0 - 7.5	0.1	-0.1	0.3	
0 - 10	0.2	0.0	0.4	
0 - 5	0.2	0.0	0.4	***
0 - 2.5	0.3	0.1	0.5	***
7.5 - 0	-0.1	-0.3	0.1	
7.5 - 10	0.1	-0.1	0.3	
7.5 - 5	0.1	-0.1	0.3	
7.5 - 2.5	0.2	0.0	0.4	
10 - 0	-0.2	-0.4	0.0	
10 - 7.5	-0.1	-0.3	0.1	
10 - 5	0.0	-0.2	0.2	
10 - 2.5	0.1	-0.1	0.3	
5 - 0	-0.2	-0.4	0.0	***
5 - 7.5	-0.1	-0.3	0.1	
5 - 10	0.0	-0.2	0.2	
5 - 2.5	0.1	-0.1	0.3	
2.5 - 0	-0.3	-0.5	-0.1	***
2.5 - 7.5	-0.2	-0.4	0.0	

Comparisons significant at the 0.05 level are indicated by ***.			
Fortification Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**	
2.5 - 10	-0.1	-0.3	0.1
2.5 - 5	-0.1	-0.3	0.1

Table 3.8 Days Comparison for microbiological properties based on Tukey's method

Comparisons significant at the 0.05 level are indicated by ***.				
Days Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
Day 28 - Day 14	0.17	0.05	0.29	***
Day 28 - Day1	1.14	1.02	1.26	***
Day 14 - Day 28	-0.17	-0.29	-0.05	***
Day 14 - Day1	0.97	0.85	1.09	***
Day1 - Day 28	-1.14	-1.26	-1.02	***
Day1 - Day 14	-0.97	-1.09	-0.85	***

It was recommended that yogurt or fermented milk should contain atleast 10^8 CFU/serving (EFSA, 2010; Zare et al., 2011) which represents one million viable cells per gram at the time of consumption. The Fig. 3.9 represents the total viable count of protein extracted from pinto and kidney bean at different fortification level with respect to storage time periods of 28 days.

From the Table 3.4, it is clear that ANOVA model was found highly significant as p-value <0.05 and R^2 value equals 0.97.

Table 3.9 Beans Comparison for microbiological properties based on Tukey's method

Comparisons significant at the 0.05 level are indicated by ***.			
Beans Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**	
Pinto - Kidney	0.06	-0.02	0.14
Kidney - Pinto	-0.06	-0.14	0.02

No significant effect has been observed on microbial properties due to fortification, days and interaction of fortification level and days (Table 3.5, 3.6, and 3.7). However, there was a significant effect due to beans type itself as main as well as its two way and three-way interaction with fortification level and days. (Table 3.5)

3.3.3. Rheological Characteristics

The rheological behavior of yogurt is influenced by a three-dimensional network formed by the protein. The enhanced milk protein content facilitated the yogurt samples to form strong protein-protein bonds (Yu et al., 2016). The power law equation was used to describe the rheological behavior of the control and fortified yogurt. The power law equation (Eq. 3.2) also applied for yogurt in other research studies.

The results for the power law constants n and K with coefficient value (R^2) are shown in Table 3.10. Determination Coefficient (R^2) above 0.90 or all the models showing satisfactory fit of flow curve. The control and fortified yogurt showed a pseudo-plastic behavior as value of $n<1$. The consistency index increased significantly with the increase in the fortification level. Kucukcetin et al. (2012) reported the similar increase in consistency index value with increase in the fortification of lentil flour in yogurt. The highest consistency index value 23.01 $Pa.s^n$ was obtained at 10% kidney bean protein

fortified yogurt. The consistency index and flow behavior index decreased with increase in the storage period. Singh et al. (2008) reported the decrease in the value of the consistency index and flow behavior index of calcium fortified yogurt during the storage studies of 14 days. Santillan-Urquiza et al. (2017) reported the decrease in consistency index value of minerals fortified yogurt during the storage. The decrease in consistency index is mainly due to the change in the gel structure, in agreement with loss of stiffness of the protein matrix (Lee & Lucey, 2010)

Flow behavior index decreased significantly with increase in the fortification level. The values varied from 0.43-0.34. The flow behavior of control yogurt obtained was 0.43 whereas the flow behavior of kidney bean protein fortified yogurt at 10% obtained was 0.32 respectively on day 1. There was decrease in the flow behavior index value from day 1 to Day 28. The flow behavior of control yogurt obtained was 0.39 whereas the flow behavior of kidney bean protein fortified yogurt at 10% obtained was 0.29 respectively on day 28. Kucukcetin et al. (2012) reported the similar decrease in consistency index value with increase in the fortification of lentil flour in yogurt whereas Santillan-Urquiza et al. (2017) reported no effect on flow behavior value of minerals fortified yogurt during the storage.

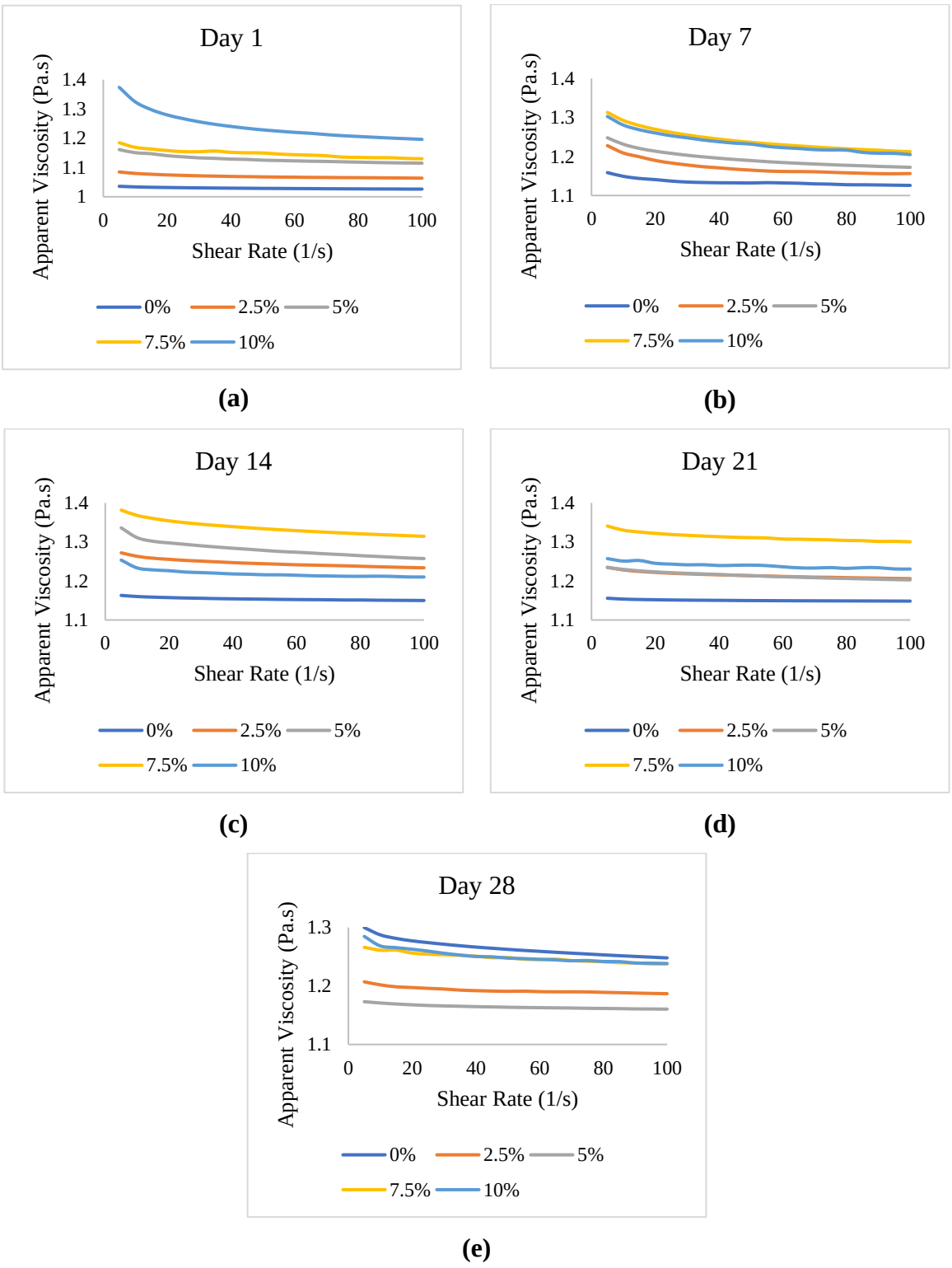


Fig. 3.6 Apparent viscosity vs shear rate curve for control (0%) and KBPC fortified yogurt during storage of 28 days (a) Day 1 (b) Day 7 (c) Day 14 (d) Day 21 (e) Day 28

The apparent viscosity with respect to time for all the fortification level during storage of 28 days were shown in Fig. (3.10-3.14). The apparent viscosity increased with increase in the fortification level and storage period. The apparent viscosity decreases with increase in the time that represents the shear-thinning behavior of yogurt. No significant difference was observed in the apparent viscosity of the control yogurt and fortified yogurt on day 1 but there was sudden increase in the apparent viscosity of fortified yogurt on day 7 as compared to control. Results shows that there was increase in viscosity with fortification, but no significant difference was obtained between groups.

Table 3.10 Rheological parameters of stirred yogurt samples.

Storage time (Days)	**1**			**7**			**14**			**21**			**28**		
Fortification Level	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**	**K**	**n**	**R^2**
Control	10.1	0.42	0.96	9.32	0.41	0.97	8.67	0.4	0.96	8.32	0.39	0.96	8.22	0.39	0.97
Kidney Bean Protein 2.5%	12.57	0.42	0.98	12	0.41	0.94	11.22	0.4	0.98	10.97	0.38	0.94	10.85	0.37	0.96
Kidney Bean Protein 5%	16.04	0.39	0.98	15.16	0.35	0.96	14.49	0.34	0.94	14.24	0.33	0.93	14.13	0.33	0.98
Kidney Bean Protein 7.5%	18.75	0.36	0.99	18.39	0.34	0.97	17.71	0.33	0.93	16.84	0.32	0.98	16.73	0.31	0.97
Kidney Bean Protein 10%	23.01	0.32	0.98	22.49	0.31	0.99	21.8	0.3	0.99	21.02	0.29	0.96	20.89	0.29	0.99

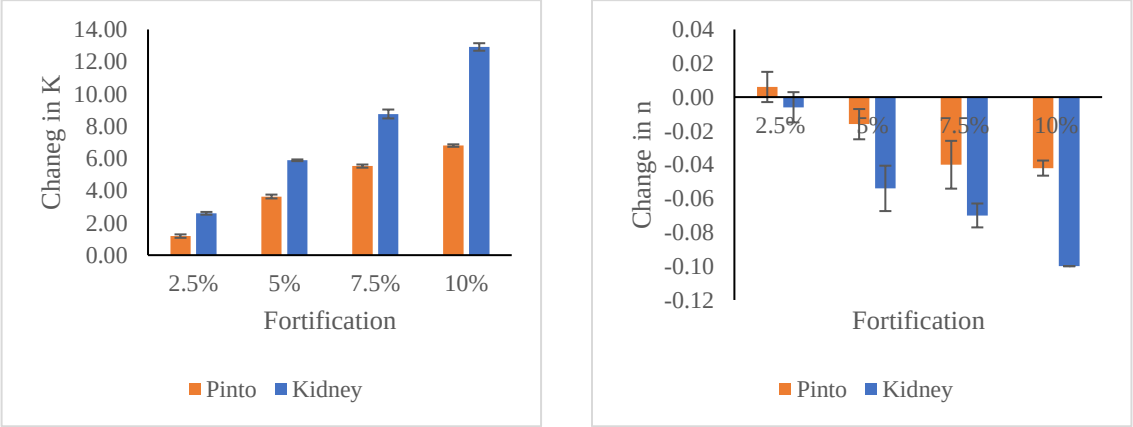
3.4. Comparison of Fortified Yogurt for Using Different Bean Types, Fortification Levels and Number of Days Based on its Physio-chemical, Microbiological and Rheological Properties

Table 3.1 and Table 3.6 represents the ANOVA procedures applied on physiochemical and microbiological properties, respectively, to know the effect of fortification levels, bean types and storage period in days. It was found that ANOVA model for both physiochemical as well as microbiological properties was significant with p-value less than 0.05 and R^2 values greater than 0.93 for both the properties.

Significant effect due to change in days and bean types, respectively, has been seen on ash content and color b*. No significant effect due to change in fortification levels, bean types and number of days (main effect) has been observed in any other physiochemical properties. Moreover, significant effect has been observed due to two-way interaction of bean types and number of days on ash content, moisture content, total solids content and color b*, whereas, three-way interaction effect has been observed in moisture content, total solids content and color b*. In rest of the physiochemical properties, no significant effect has been obtained due to two-way or three-way interaction of fortification levels, bean types and days.

Similarly, microbiological properties have also been tested using ANOVA procedure (Table 3.6). Change in bean types and its interaction with change in fortification level and number of days was found to have significant impact on microbial properties of yogurt.

Comparison of the rheological properties between pinto and kidney beans fortified yogurt is shown in Fig. 3.7 The value of K at different fortification level with respect to control has been increasing for both pinto and kidney bean fortified yogurt. The values of K were found to be significantly higher for the yogurt fortified with kidney beans compared to pinto beans. Similar results have been observed in case of n but trend was found to be decreasing. On comparing both the beans, the value of n was found to be significantly smaller for yogurt fortified with kidney beans than with pinto beans.



(a) **(b)**

Fig. 3.7 Change in value of K and n of yogurt fortified with pinto and kidney beans at different fortification levels with respect to control

3.4.1. Fortification Comparison Based on Tukey's Test

Like ANOVA, Tukey's method was applied to investigate the significant difference between the mean values of all physio-chemical and microbiological properties. Mean value of moisture content from different samples were significantly different among all the fortification level. Results based on Tukey's method for comparison of physiochemical properties among different levels of fortification,

significant differences have been observed in mean values of fat, protein, pH, TA, WHC and Color *L among different fortification levels. Significant difference has been observed in ash content and color a* among all other fortification levels, except between 7.5% and 10% fortification. Mean values of color b* from different samples are significantly different between 0% and all other fortification levels i.e., 5%, 7.5% and 10%. (Table 3.2)

Similarly, microbial properties were also assessed using Tukey's method. Significant differences were observed among the means values of total viable count of yogurt fortified at 0%, 2.5% and 5%. (Table 3.7)

3.4.2. Beans Type Comparison Based on Tukey's Test

To estimate the significant difference for using different beans type in fortification of yogurt, Tukey's methods was also applied on different physio-chemical and microbiological properties. The mean values of ash content, fat content, moisture content, pH, WHC, color a* were found to be significantly higher for pinto beans compare to kidney beans protein concentrate by 0.06, 0.11, 0.63, 0.05, 2.07, and 1.20%, respectively, whereas, the means of protein content, total solids content, titratable acidity, color L* and color b* were found to significantly lower by 0.88, 0.63, 0.02, 2.35, and 0.18% in pinto beans (Table 3.11).

However, no significant difference has been observed pinto and kidney beans in microbial properties.

3.4.3. Days Comparison Based on Tukey's Test

On comparing physio-chemical properties between days, no significant difference has been observed among any day for ash content, whereas, significant difference has

been observed among all the days for protein content, and different colors. Except between Day 1 and Day 28 in moisture content and total solids content, and between Day 1 and Day 7 in WHC, no significant difference has been observed in any of days for remaining physio-chemical properties. (Table 3.4 and Table 3.5).

However, on analyzing microbial properties, significant differences has been observed in mean values of total viable counts among all storage period (days) (Table 3.8)

Table 3.11 Proximate analysis of pinto, kidney, and commercial bean protein powder

Analysis	Pinto Bean Protein Powder Dry mater (g/100g)	Kidney Bean Protein Powder Dry mater (g/100g)	Commercial Legumes Protein Powder Dry mater (g/100g)
Crude Ash Content	4.688	3.874	3.874
Moisture Content	5.45	5.63	5.73
Crude Fat	1.94	1.38	1.36
Crude Fiber	0.53	0.48	0.04
Crude Protein	79.74	82.24	85.28
Starch Content	0.00	0.00	0.00
Color L*	76.50	79.49	82.56
Color a*	6.17	2.87	3.12
Color b*	26.38	13.24	14.25

3.5. Conclusions

The ANOVA procedures applied on physiochemical properties for pinto and kidney bean protein concentrate fortified yogurt are found to be statistically significant with p-value less than 0.05 and R^2 values to be more than 0.93 for all the physiochemical

properties. The effect of fortification of yogurt using protein concentrate of kidney and pinto bean on all the physiochemical properties are found to be significant.

The moisture content of fortified yogurt (using both kidney as well as pinto beans) decreased with increase in fortification level that implies increased in TSC with respect to fortification level. There were no significant differences found on moisture content and TSC during the storage of 28 days expect between Day 1 and Day 7 for both bean types fortified yogurt. The protein content of fortified yogurt (using both the beans) was found to be higher than the control yogurt. The maximum TA and minimum pH observed at the 10% fortification level on Day 28. The water holding capacity for both the bean types increased with increase in fortification level but decreased with respect to storage period of 28 Days. The color of PBPC and KBPC fortified yogurt increased with increase in the fortification level but there was no significant difference found between control and 2.5% fortified yogurt on Day 1. Based on the Tukey's comparison between different bean types fortification on the physiochemical properties, it was found that yogurt fortified with KBPC is high in protein content, total solids content and titratable acidity. The pH value of KBPC fortified yogurt is lower with a difference of 0.02. The color of KBPC is lighter as compared to PBPC but the water holding capacity of PBPC fortified yogurt is more with a difference of 2.12.

The ANOVA model for microbiological properties was found significant. On comparing the mean values for different fortification levels, it was found that control yogurt (0%), 2.5% and 5% are statistically different for microbiological properties. No significant difference has been observed in the means values of viable count between different bean types. All the days are found to have significant difference for viable

counts. The power law equation is used to describe the rheological behavior of the control and fortified yogurt. It is found that the consistency index values increased with increase in fortification level whereas the flow behavior values decreased. The flow behavior of PBPC fortified yogurt is more than the KBPC fortified yogurt. Results shows that there was increased in viscosity with fortification, but no significant difference was obtained between groups after 21 days of storage period.

On comparing both PBPC and KBPC fortified yogurt, KBPC fortified yogurt was found to be better in terms of protein content, total solids content, color, consistency and flow behavior index.

Chapter 4

Summary and Conclusions

This research aims to evaluate the effect of fortification of different legumes protein concentration on the physiochemical, microbiological and rheological characteristics of yogurt.

The PBPC fortified yogurt obtained shows the significant difference as compared to control yogurt. The physiochemical properties of PBPC fortified yogurt are higher in protein, total solids, and ash content as compared to control yogurt. The pH and TA of the fortified yogurt obtained. The protein content ranges from 5.87 to 10.14 with respect to increase in fortification from 2.5 to 10 %. The fortification of yogurt with PBPC increases the WHC of yogurt by 1.70, 2.03, 3.01 and 5.12 % with an increase in fortification from 2.5 to 10% as compared to control yogurt samples. The color of PBPC fortified yogurt increase with increase in the fortification levels. There was no significant effect obtained on the microbiological properties. Addition of PBPC to yogurt increases the viscosity as compared to control sample. However, both control and fortified yogurt show a shear-thinning behavior.

The KBPC fortified yogurt also found to be significant for all the physiochemical properties. KBPC fortified yogurt also shows a significant increase in protein, total solids, ash, WHC, TA with the fortification of yogurt. Both decreases in pH and increase in TA shows the better acidification of yogurt fortified with KBPC as compared to control yogurt sample. No significant effect observed on microbiological properties of

fortified yogurt due to the addition of KBPC. Both control and KBPC yogurt samples show a shear thinning behavior with $n<1$.

On comparing the KBPC and PBPC yogurt, it was obtained that PBPC fortified yogurt is lower in protein content, total solids content and titratable acidity as compared to KBPC fortified yogurt samples. The pH value of PBPC fortified yogurt is higher than the KBPC fortified yogurt with a difference of 0.02. The color of PBPC fortified yogurt is darker as compared to KBPC but the water holding capacity of PBPC fortified yogurt is higher with a difference of 2.12. No significant difference found between beans types on microbiological properties. Both fortified yogurts show a shear thinning behavior with increase in viscosity during storage period of 28 days. The value of K at different fortification level with respect to control has been increasing for both pinto and kidney bean fortified yogurt. The values of K were found to be significantly higher for the yogurt fortified with kidney beans compared to pinto beans.

Based on the results it can be concluded that protein concentrate from legumes can be used as a fortification source to develop an alternative fermented dairy product with good nutritional values, without having an effect on microbiological properties. However, there was some effect on rheological properties due to the fortification of different type of protein which could affect the yogurt structure.

On comparing both beans types, KBPC fortified yogurt was found to be better in terms of protein content, total solids content, color, consistency and flow behavior index.

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Chapter 5

Recommendations for Future Work

In the future, detailed studies of microbiological properties could be conducted to see the prebiotic effect of extracted protein from different bean types on the growth of *Lactobacillus bulgaricus and Streptococcus thermophilus* during a storage period of 28 days. Textural properties and protein digestibility of the fortified yogurt samples will help in better understanding the overall quality of the fortified yogurt. Sensory analysis can be carried out in the future to determine the consumer acceptability.

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