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Fortification of Yogurt with Chickpea Flour Enhances Overall Quality of Yogurt

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Xi Chen

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Fortification of Yogurt with Chickpea Flour

Enhances Overall Quality of Yogurt

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By

Dr. Kanika Bhargava

Committee Chairperson

Dr. Tawni Holmes

Committee Member

Dr. Ranjith Ramanathan

Committee Member

To my father, Jianhua Chen,

and to my mother Lili Han

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ABSTRACT

Yogurt is well known for its health promoting properties. Chickpea flour has been suggested to increase the growth of probiotic bacteria during yogurt production, however, studies on the effects of dried beans flour on physico-chemical characteristics of yogurt are limited. This study aimed to evaluate the effects of chickpea flour on the overall quality of yogurt and anticipate inventing a new protein rich yogurt product. In this study, 2% low-fat milk was supplemented with 1%- 5% (w/v) chickpea flour, inoculated with a yogurt culture (Danisco YO-MIX 883 LYO 500 DCU), fermented and stored at 4 ºC. The control and fortified samples were analyzed for pH, total titratable acidity (TTA), viscosity, and microbial counts over a 21-day storage period (4°C). The color test, viscosity, and sensory evaluation was also performed at the initial day of production.

Results demonstrated that chickpea flour stimulated the growth of *Streptococcus thermophiles* and *L. delbrueckii subsp. Bulgaricus* after the initial fermentation and maintained a greater bacterial counts over a 21-day refrigerated storage. Furthermore, there was a significant ($p<0.05$) decrease in pH and enhancement in TTA in chickpea flour containing yogurt across the whole storage period. Fermentation time was decreased by1.5 hours with inclusion of chickpea flour (pH:4.5). The color and viscosity value were almost constant and there was not a significant ($p<0.05$) difference between the control sample and the fortifying samples. There were similar results on sensory properties comparing the plain yogurt and yogurt with 1% and 2% of chickpea flour.

The results suggest that chickpea fortified yogurt offers an alternative new fermented dairy product and provides a better quality.

Keywords: Yogurt, chickpea, probiotic, prebiotic, physico-chemical, sensory, *Streptococcus thermophiles, L. delbrueckii subsp. Bulgaricus*

CHAPTER ONE: INTRODUCTION

1.1 Introduction and Statement of the Problem

Yogurt has been considered to be one of the most popular fermented dairy food in the United States and worldwide (Soccol, Prado, Garcia, Rodrigues, & Medeiros, 2014). Yogurt can not only reduce symptoms caused by lactose maldigestion, it is a good source of several micronutrients and it may promote diet quality and boost metabolism as an energy-balanced dairy product (Morelli, 2014). Yogurt is gaining significant popularity in the last two decades. In addition, yogurt consumption is associated with the improvement of living standard and the enhancement of health consciousness (Wang, Livingston, Fox, Meigs, & Jacques, 2013).

The chickpea (Cicer arietinum L.) is a kind of protein rich legume and it's also a good source of micronutrients (selenium, iron, zinc, calcium, magnesium, potassium, copper, and phosphorus) (Thavarajah, 2012). Importantly, it is low in fat, high in protein, rich in prebiotics, and exhibits a low glycemic index. Therefore, it lowers the risk of diabetes, cardio vascular issues and obesity (Mudryj et al., 2014). In addition, it showed a good potential antioxidant ability (Kou, Gao, Xue, Zhang, Wang, &Wang, 2013). According to The Dietary Guidelines of USDA, a person should consume at least 3 cups of legumes each week (Gebhardt et al., 2008).

Pulses are underutilized in the American diet. The concept of utilization of legumes with probiotics may satisfy human food and fiber needs and increase agriculture and natural resource sustainability. Application of chickpea as an ingredient in yogurt provides new opportunities for increasing quality and shelf life of probiotic products such as yogurt. It has the potential to improve nutrition as a part of dairy supplements (Newby, 2009).

There is an excellent potential to develop chickpea flour supplemented yogurt and probiotic products. Chickpeas are rich in prebiotics including the raffinose-family oligosaccharides (RFO), resistant starches and fibers (Johnson et al., 2013). Pulse ingredients could serve as prebiotic and nutritional source for yogurt and probiotic products. Functional oligo-saccharides present in pulses also offer health benefits on gastro intestinal health (Guillon and Champ, 2002). Besides, health benefits of yogurt, its physical properties, appearance and texture are important for consumer acceptability. Addition of ingredients (e.g. milk solids, stabilizers, prebiotic, milk protein, calcium, and fibers) and modifying process conditions are common practices to increase overall quality and shelf life of yogurt (Zare et al., 2011). Last but not least, most of the bacteria do not grow rapidly in milk. Therefore, it will take more fermentation time to process yogurt products (Roy, 2005). Accelerated fermentation process and a more effective yogurt manufacturing process need to be investigated, if pulses are used.

Over the past several years, a number of strategies to improve the prebiotic content of yogurt have been investigated. Common ingredients rich in prebiotics such as gum arabic, wheat bran, dietary fiber, and insulin have been investigated (Charalampopoulos et al., 2002; De Souza Oliveira et al., 2009; Seckin and Ozkilinc, 2011). Addition of lentil flour in yogurt has recently being investigated by a research team in Canada (Zare et al., 2012a; Zare et al., 2012b). Therefore, the proposed study will focused on evaluation of supplementing chickpea flour on physiochemical, textural, sensory quality and shelf life of yogurt.

1.2 Hypothesis and Objectives of the Study

1.2.1 Hypothesis: Milk supplementation with chickpea flour will offer an alternative as a new product and provide better quality yogurt.

1.2.2 Objectives and Specific Aims: To formulate and apply chickpea flour to increase overall quality and shelf life of yogurt, this study aims to:

1. figure out the optimum concentration of chickpea flour that can be added in yogurt

2. investigate the effects of yogurt supplementation with chickpea flour on pH, titratable acidity, microbial counts, and viscosity during a 21 days of storage, a sensory evaluation and color of the final product.

CHAPTER TWO: REVIEW OF THE LITERATURE

2.1.1 Statistics and Facts on the Current Yogurt Market

Yogurt is categorized as a dairy product (Christopher G. Davis & Owens, 2010) and is manufactured from the bacterial fermentation of milk. It is a delicious food or snack widely used across the globe. Although yogurt has a relatively short history in the United States, it has no doubt been a significant part of the U.S. dairy market. The per capita consumption of milk has been declining since 1975, while the consumption of yogurt is on the rise (USDA ERS, 2015). A 2015 USDA ERS study found that the per capita consumption of yogurt in the United States was about 14.9 lbs per year in 2013, which increased more than 7-fold between 1975 and 2013. Currently, yogurt has a very positive reputation and people think that it's a healthy food. However, we find that most people in the U.S. don't eat nearly as much as yogurt as people around the world. American's yogurt consumption is still very low compared to annual consumption of 78.7 lbs per person in parts of Europe in 2013 (Research, 2013). According to the data from Statista Inc. (2015), the U.S. yogurt sales increased 7.6% by 2013. While still in its infancy the U.S. yogurt market has the potential to make climbing sales from 7.3 billion dollars in 2012 to 9.3 billion dollars by 2017 (The Statista Inc.).

2.1.2 History of Yogurt

Food historians generally agree that yogurt and other fermented milk products originated in the Middle East around 10,000 B.C. It was initially made by leaving milk out in the sun to ferment it spontaneously, which grew out the whole process for preserving milk. People continued the practice not only did it prolong the shelf life of milk, but also people preferred the taste. Evidence has shown that people has immigrated it around 5,000 B.C. (Tamime, 2008). As ancient as it is, yogurt production did not become commercialized and standardized until 1900s. Since that time, the popularity and consumption of yogurt has been tremendously. Due to the highly praised health benefits, a variety of innovative yogurt products appeared on the market with the high demand of consumers, as well as improved texture, flavor, and nutritional characteristics (Foerst & Santivarangkna, 2016).

Yogurt is defined as the food produced by culturing one or more of the optional dairy ingredients with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and Streptococcus thermophiles (21 C.F.R. § 131.200 2015). Right now, the general category of yogurt consists of firm yogurt, stirred yogurt, drinkable yogurt, and frozen yogurt (Chandan & O’Rell, 2006). Most of yogurt in the U.S. is low fat or reduced fat yogurt. Nonfat yogurt products are usually fortified with some kind of protein as a fat replacer, such as whey protein (Sodini, Montella, & Tong, 2005).

2.1.3 Nutrient Facts and Benefits of Yogurt

Nutrition	Unit	Cup (8 fl oz) 245g
Energy	kcal	149
Protein	g	8.50
Total lipid (fat)	g	7.96
Carbohydrate	g	11.42
Sugar, total	g	11.42
Fiber, total dietary	g	0.0
Calcium, Ca	mg	296
Iron, Fe	mg	0.12
Zinc, Zn	mg	1.45
Copper, Cu	mg	0.022
Magnesium, Mg	mg	29
Phosphorus, P	mg	233

Potassium, K	mg	380
Sodium, Na	mg	113
Fatty acids, total saturated	g	5.135
Vitamin A, RAE	µg	66
Vitamin B-12	µg	0.91
Vitamin C, total ascorbic acid	µg	1.2
Vitamin D (D2 + D3)	µg	0.2
Vitamin E (alpha-tocopherol)	mg	0.15
Thiamin	mg	0.071
Riboflavin	mg	0.348
Niacin	mg	0.184

Table 1. Yogurt, Nutrition Facts. (plain, whole milk)

Source: USDA National Nutrient Database for Standard Reference,

www. nal.usda.gov/fnic/foodcomp/search/

2.1.4 Beneficial Effects of Yogurt

Yogurt not only tastes good, but also offers health benefits. As can be seen in Table 1., one-cup (8 oz.) serving of whole milk plain yogurt contains 296mg of calcium, plus about 8.5g of high quality protein (approximately 20 % of the daily recommended value) and is an excellent source of potassium, phosphorous, magnesium, zinc and vitamins B2 (riboflavin), B3 (niacin), B6 and B12. Besides, it is relatively low in calories (U.S. Department of Agriculture, 2015). Vitamins and minerals are essential for our body functions and metabolic reactions. The large amount of calcium and Vitamin D in yogurt is important for bone mineralization. Yogurt has been considered to improve the bone density and reduced the risk of hip fracture and osteoporosis, which are believed to be a positive association with the high calcium and protein content (Rizzoli, 2014). Besides, the high content of protein and calcium is associated with reduced risk of body fat gain and obesity as well as cardiovascular disease (Astrup, 2014). The potassium may help flush out excess sodium from our system thereby helping to lower blood pressure level (Health, 2006), and then reduce the risk of cerebrovascular accident and myocardial infarction (Houston, 2011). Zinc and magnesium are cofactors for many metabolic functions. There is a relation between a higher intake of magnesium and reduced type 2 diabetes (Song, Manson, Buring, & Liu, 2004). B vitamins play key roles as coenzymes and precursors of metabolic processes (Gropper & Smith, 2012). In addition, several proteins, peptides, and minerals contained in yogurt have an antihypertensive effect (Usinger, Ibsen, & Jensen, 2009). Besides, consumption of yogurt contributes to a high satiety rating of individuals and lower body fat (Keast, Hill Gallant, Albertson, Gugger, & Holschuh, 2015).

Beyond conservation of these vital basic nutrients of milk and offering a good taste, we can find the live and active cultures that were produced by the fermentation process providing additional health benefits thereby enhancing the overall quality of yogurt.

The science consistently recognized the health effects of yogurt, which improve lactose digestion (de Vrese et al., 2001) and eliminate symptoms caused by lactose maldigestion (Morelli, 2014).

There is a trend of adding probiotics for providing additional health benefits due to the inability of Lactic acid bacteria surviving in low acidic environments such as the human gastrointestinal tract (Foerst & Santivarangkna, 2016).

2.1.5 Probiotics

The live and active culture that are usually added in yogurt for the health effects are probiotics which are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” by the Food and Agriculture Organization of the

United Nations World Health Organization (2001). Currently, 95% fermented milk products within the market contain probiotics. A number of bacteria are used as probiotic, including specific strains of lactobacillus and Bifidobacterium, such as *Lactobacillus delbrueckii subsp. Bulgaricus* (Guarner et al., 2005).

Probiotics are good bacteria that play an essential role in our digestive, metabolic, and immune function. They produce enzymes that break down foods, regulate vitamin and nutrient uptake and convert sugar into essential nutrients. Probiotics are also the first line of defense for our immune system. The probiotics can survive and be active in the gastrointestinal tract thereby they help create a balanced environment in our gut, which will help us maintain the overall health. A good balance of gut bacteria is crucial for good digestive health, which makes yogurt loaded with probiotics in turn directly affects our immune system and our ability to fight off foreign invaders (Balakrishnan & Floch, 2012). Moreover, the probiotic in yogurt can play a role in lowing blood pressure and promoting heart health (Astrup, 2014).

The scientific recognized health benefits of fermented milk-based probiotic preparations include reduction in Helicobacter pylori infection (Sachdeva & Nagpal, 2009) (Yang & Sheu, 2012), improvements in irritable bowel syndrome (Lee et al., 2013) and inflammatory bowel disease (Jonkers, Penders, Masclee, & Pierik, 2012), prevention of antibiotic-associated diarrhea (Hempel et al., 2012), alleviation of constipation (Miller & Ouwehand, 2013), prevention in gastric cancer (Cousin, Jouan-Lanhouet, Dimanche-Boitrel, Corcos, & Jan, 2012), and affection of part brain regions activity (Tillisch et al., 2013).

Currently, there is a lot of emerging evidence showing the health benefits of yogurt. Yogurt is considered to be healthy in many ways due to being rich in nutrients. Yogurt consumption was associated with a better diet quality and healthier metabolic profiles (Wang, Livingston, Fox, Meigs, & Jacques, 2013). If we can make the public as well as health care practitioners even more aware the health benefits of yogurt, we can encourage more Americans to enjoy this healthy delicious food. The International Dairy Foods Association (IDFA) reinforced the importance of including dairy products, saying milk, cheese and yogurt are valuable sources of nutrients, especially protein (International Dairy Foods Association, 2014). In addition, it is suggested that increasing the intake of yogurt as a nutrient-dense food will be the most beneficial (Olson, R., Casavale, K., Rihane, C., Stoody, E. E., Britten, P., Reedy, J., . . . Rodgers, A. B., 2016).

2.1.6 Probiotics and Prebiotics

Owing to the potential of providing a wide range of health benefits and the increasing awareness of individuals, there are a variety of novel yogurt products on the basis of flavor, texture, types, package, processing, ingredients (Yildiz, 2009). Apart from the conventional yogurt ingredients, probiotics and prebiotics have been proven to provide beneficial effects and used extensively in the yogurt industry. Gibson and Roberfroid (Glenn & Roberfroid, 1995) first defined prebiotic as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. At present, according to the criteria of prebiotic, inulin and oligofructose meet the standards. More studies are promising and limited in discovering other candidates, such as galactooligosaccharides, lactulose, isomaltooligosaccharides, lactosucrose, and xylooligosaccharides. They have shown the ability of stimulating the number of lactobacilli and especially bifidobacteria (Roberfroid, 2007).

Several studies reported a positive effect with the addition of prebiotic ingredients in terms of probiotic counts, sensory evaluation, rheological and physicochemical properties (De Castro, Cunha, Barreto, Amboni, & Prudencio, 2009) (Oliveira et al., 2009) (Arcia, Costell, & Tárrega, 2011) (Gonzalez, Adhikari, & Sancho-Madriz, 2011). The results of another research showed that with the adding of oligofructose, yogurt has a weak gel character while there was no significant effects on the pH, proteolysis or the growth of *Streptococcus hermophilus* or *Lactobacillus bulgaricus* during 28 days of refrigerated storage (Cruz et al., 2013).

In different parts of the world, these fermented foods are used as an ingredient in cooked products or are consumed as a condiment, snack, dessert, drink or spread. Factors such as source of milk (from different animals), diverse microflora, addition of supplements and preservative and the processing techniques like freezing, drying and concentration contribute to the variations in fermentation of milk.

2.2.1 Chickpeas

Chickpea (Cicer arietinum L.) is categorized as a pulse, a subgroup of grain legume. Legume refers to the plants whose fruit is enclosed in a pod, while pulses represent only the dried seed (Grusak, M., 2005). The major legumes include dry peas, lentils, chickpeas, soybeans, fava beans, peanuts, lupins, and beans, among which peas, beans, lentils, and chickpeas belong to pulses (USA DRY PEA & LENTIL COUNCIL, 2010).

The production of chickpea is approximately two times more than that of lentils worldwide. Besides, chickpea is second in consumption worldwide after dry beans as human food (USA

DRY PEA & LENTIL COUNCIL, 2010). As one of the earliest cultivated vegetables on earth (USA DRY PEA & LENTIL COUNCIL, 2010), chickpea has ranked the third most important grain legume in the world on the basis of total grain production (FAO, 1994), yet is underused in the United States. The United States stated chickpea production in 1981, which was over 60 years later than the initial production of lentil and dry pea. Today, nearly 75% of the United States chickpea production are for exportation whereas in the domestic market chickpeas are commonly used in salad as canned, ground flour for baking and paste for *Hummus* (USA DRY PEA & LENTIL COUNCIL, 2010).

2.2.2 Nutrient Facts of Chickpea

Chickpeas, ¼ cup dry

Nutrition Facts

Calories 180	Calories from Fat 25

	% Daily Value*
Total Fat 3g	5%
Saturated Fat 0g	0%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 10mg	0%
Potassium 438mg	13%
Total Carbohydrate 30g	10%
Dietary Fiber 9g	36%
Sugars 5g	
Protein 10g	

Vitamin A 0%	Vitamin C 4%
Calcium 6%	Iron 15%
Thiamin 16%	Vitamin B6 13%
Folic Acid 70%	Phosphorus 18%
Magnesium 14%	Zinc 11%
Copper 21%	Manganese 55%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300mg	375mg
Dietary Fiber		25g	30g

Calories per gram
Fat 9 · Carbohydrate 4 · Protein 4

Figure 1. Chickpea Nutrition Facts Panel

Source: USA DRY PEA & LENTIL COUNCIL, http://www.pea-lentil.com/technical-manual.

Chickpeas, like other legumes, are an excellent source of carbohydrates, protein, dietary fiber, vitamins, and minerals, and has become a popular food for vegetarians and individuals who have inadequate intake from animal proteins. Compared with the other pulses, chickpea provides a higher value of total carbohydrate (Jukanti, Gaur, Gowda, & Chibbar, 2012).

Chickpeas contain a large amount of nutrients, including 22% protein, 8% dietary fiber, and only 5% fat (USA DRY PEA & LENTIL COUNCIL, 2010). In addition, chickpea is cholesterol free (Jukanti et al., 2012) and the fat is mostly unsaturated fatty acids. Even more remarkably, chickpea contain numerous dietary fibers. One cup of cooked chickpea can provide 12.46g dietary fiber, which accounts for 50% of the dietary reference intakes.

2.2.3 Dietary Fiber of Chickpea

Dietary fiber is the edible portion of plant or their extracts, which is indigestible in the human small intestine while can be fermented in the large intestine. Dietary fiber is composed of polysaccharides, oligosaccharides, and lignin (Dhingra, Michael, Rajput, & Patil, 2012). The two major components of dietary fiber are soluble fiber and insoluble fiber. Soluble fiber dissolves in water and slows digestion. However, insoluble fiber, undissolved in water, accelerates digestion and provides support to metabolic fermentation of colonic microflora, such as probiotics. Both the insoluble fiber and soluble fiber have the credit of prebiotic activity. Chickpea dietary fiber is mostly made up of insoluble fiber whereas it has a higher amount of soluble fiber among pulses (Tosh & Yada, 2010).

2.2.4 Chickpeas and Prebiotics

Chickpea contain a higher concentration of total oligosaccharides, about 144.9 mg/g, among pulses. The oligosaccharides of chickpea are mainly composed of raffinose, ciceritol, and staehyose (Han & Baik, 2006). Raffinose and stachyose that present in chickpeas, which belong to the raffinose family of oligosaccharides, are considered a good source of prebiotics. These water soluble carbohydrates pass undigested in the upper part of the gastrointestinal tract and fermented by the microflora in the large intestine (Swennen, Courtin, & Delcour, 2006).

Over the previous two decades, various oligosaccharides of natural sources have been credited with a natural prebiotic property. There is growing consideration of the bioactive potential and health promoting effect according to host metabolism and colon microflora (Brownawell et al., 2012).

Both animal studies and in vitro studies have proved the health promoting benefits of oligosaccharides consumption, such as inulin, oligofructose, lactulose, and resistant starch, including the facilitation of Bifidobacterium. The addition of raffinose family oligosaccharides had a positive influence on the survival of *Bafidobacterium lactis Bb-12 and L. acidophilius La-5* (Martinez-Villaluenga, Frías, Gómez, & Vidal-Valverde, 2006). Hernandez et al. (2012) suggested that galactooligosaccharides was an excellent supplement for stimulating the growth and improved the survival of probiotic *Lactobacillus* strains. More researches is needed to demonstrate the relationship between health outcomes and consumption of more potential prebiotics in the human body, as well as other probiotic stimulating ability (Slavin, 2013).

2.3 Application of Pulses as Ingredients in Yogurt

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 were 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). 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 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). Though the chickpea fortified yogurt has been studied, more research is needed to explore its potential on fermented dairy products.

CHAPTER THREE: METHODOLOGY

3.1 Materials

The experiment was mostly conducted in the laboratory of the Department of Human Environmental Science, the University of Central Oklahoma, Edmond, OK. Color test was done in the Department of Animal Sciences, Oklahoma State University, Stillwater, OK. 2% fat milk was purchased from the local market. Whole chickpeas (Garbanzo beans) were obtained from Associated Whole Grocers, Inc. (Kansas City, KS, USA). Materials used in these experiments were U.S.A ASTM E11 standard testing sieve (Advantech Manufacturing, New Berlin, WI), starter culture (YO-MIX 883 LYO 500 DCU, Danisco USA Inc.), water bath (WB10, PolyScience), convention oven (MO1440A-1, Thermo Fisher Scientific Inc.), ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.), magnet stirrer (MS-H280-Pro, Scilogex, LLC.), digital refractometer (HI 96801, HANNA instruments Co., Ltd), pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd), Sodium Hydroxide (NaOH) (FisherChemical, Inc.), phenolphthalein (Ricca Chemical, Inc.), MRS agar (bioWORLD, Inc.), M17 agar (Becton, Dickinson and Company), TWEEN®-80 (Sigma-Aldrich, Inc.), lactose (FisherChemical, Inc.), anaerobic chamber (FisherScientific, Inc.), digital rotary viscometer (Model NDJ-9S) , HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV).

3.2 Preparation of chickpea flour

Whole chickpeas (Garbanzo beans) were obtained from Associated Whole Grocers, Inc. (Kansas City, KS, USA) and ground to a particle size of 1.5 mm using a U.S.A ASTM E11 standard testing sieve (Advantech Manufacturing, New Berlin, WI).

3.3 Preparation of yogurt culture

Pasteurized, homogenized 2% fat milk which was collected from local market was used for yogurt processing. Yogurt culture containing *S. thermophiles* and *L. delbrueckii subsp. Bulgaricus* (YO-MIX 883 LYO 500 DCU) was from Danisco USA Inc. (Madison, WI, USA). The culture was obtained in freeze-dried form, packaged in laminated foils. They were stored at 4°C until use. The experimental protocol used for yogurt supplementation and production are shown in flow chart.

The yogurt culture was made according to the instruction of Danisco USA Inc. (Madison, WI, USA). 2% fat milk purchased from local market was pasteurized at 90°C for 10 minutes with the general purpose water bath (WB10, PolyScience), and tempered to 42°C before use. The lyophilized yogurt starter culture was weighed, and dissolved in the sterilized milk. 0.002g of yogurt starter culture was inoculated into 100 mL treated milk. The inoculated milk was incubated with a convention oven (MO1440A-1, Thermo Fisher Scientific Inc.) at 42°C for about 6 hours and stopped until the pH reached 4.5. The yogurt culture was stored at 4°C and used in 5 days (R. I. Dave & Shah, 1997).

3.4 Preparation of sample

Section 1

2% fat milk was stirred and heated to 90°C. When it cooled to 42°C, added in 1g/100mL of yogurt culture. Concentrations of 1%, 2%, 3%, and 5% of raw chickpea flour (0-5%, w/v) was added. Inoculated milk and control sample were incubated for 6 hours with a convention oven (MO1440A-1, Thermo Fisher Scientific Inc.) and stored at 4°C.

Section 2

2% fat milk and yogurt mix with the supplement of chickpea flour (1%-5% W/V) was stirred for 3 mins at 1500rpm. Homogenize for 3 mins at 70 amplitude with an ultrasonicator (QSONICA, Q700, Hudson Fusion LLC.). Pasteurised to 90°C (WB10, PolyScience) and cooled it down to 42°C. Inoculated with 1g/mL yogurt culture and incubation at 42°C for 4.5 hours, 6hours or until final pH reached, and stored at 4°C.

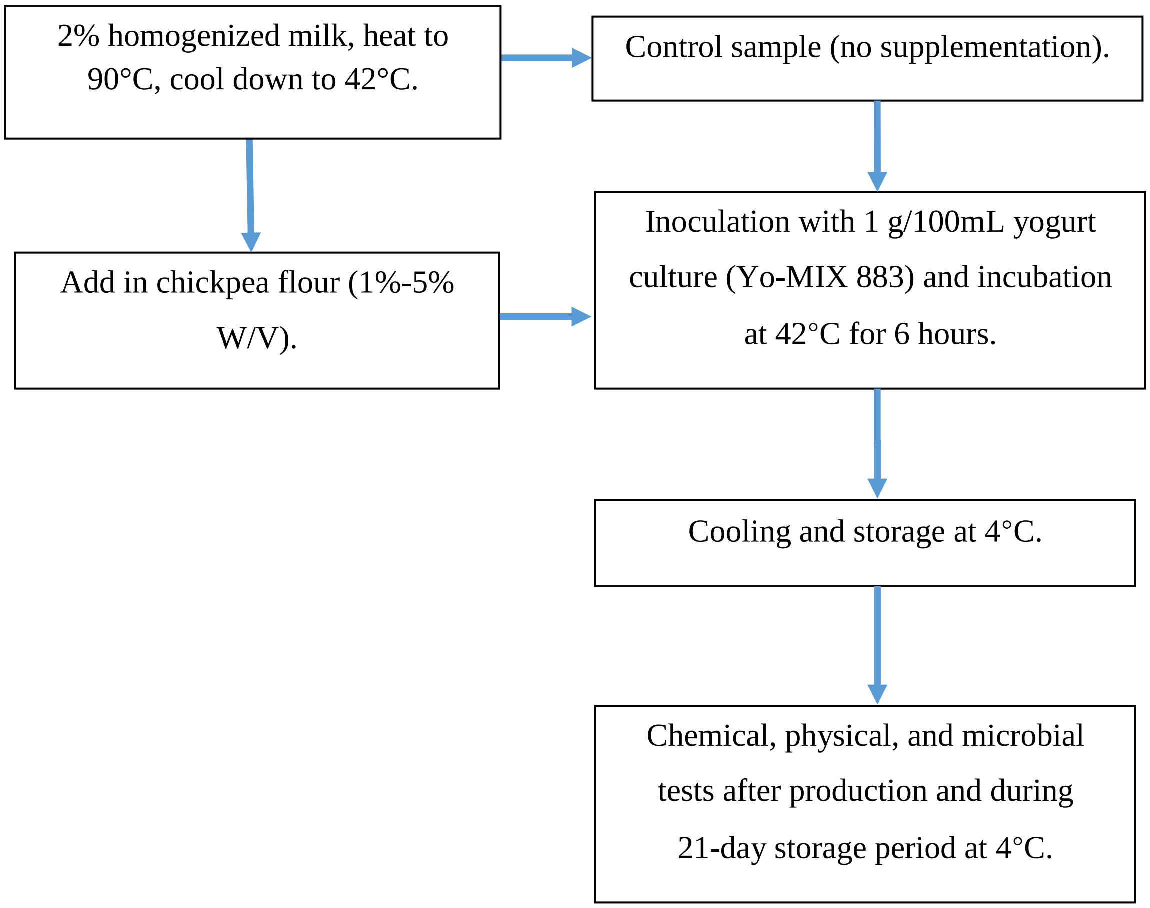

Figure 2. Schematic presentation of the process used for the yogurt supplemented with chickpea flour (1%-5% W/V) and control yogurt in section 1.

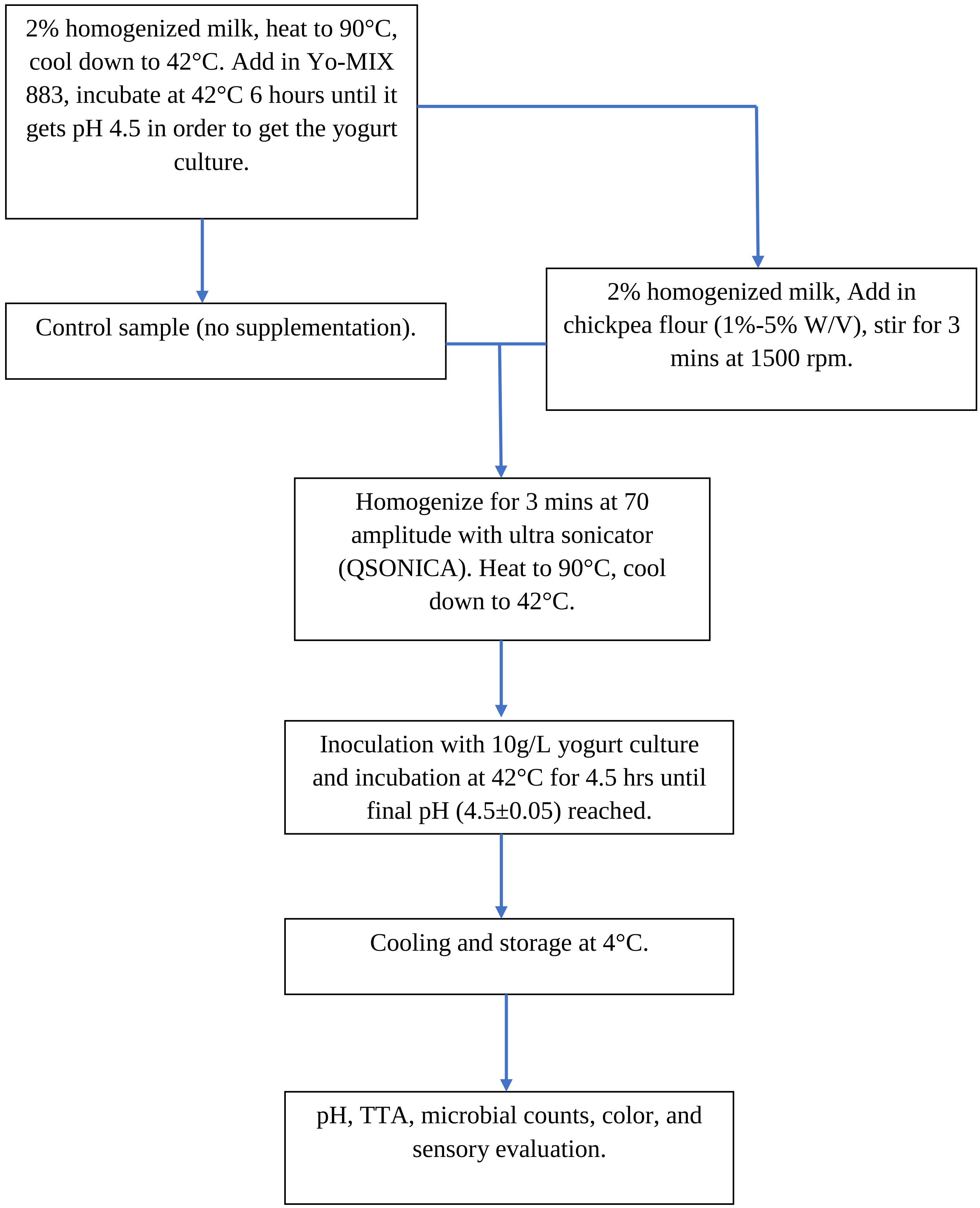

Figure 3. Schematic presentation of the process used for the yogurt supplemented with chickpea flour (1%-5% W/V) and control yogurt in section 2.

Figure 4. Convention oven (MO1440A-1, Thermo Fisher Scientific Inc.)

Figure 5. Water bath (WB10, PolyScience)

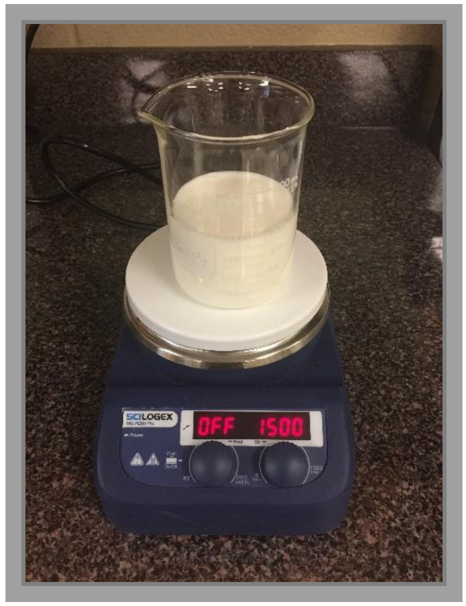

Figure 6. Magnet stirrer (MS-H280-Pro, Scilogex, LLC.)

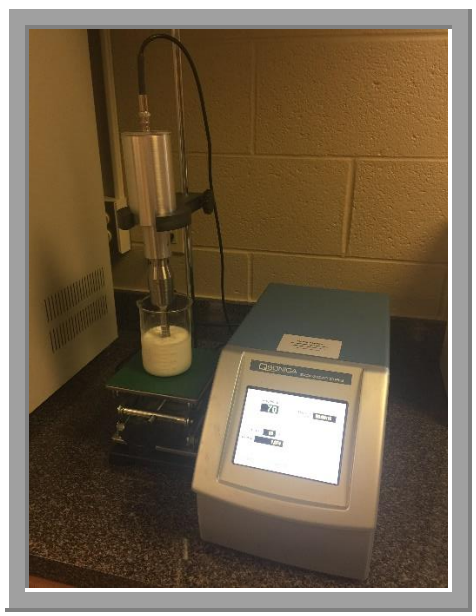

Figure 7. Ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.)

3.5 pH

The pH was determined in triplicate according to method of the AOAC (1990). The pH was measured at room temperature (21^{0}C) with a pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd). The pH meter was calibrated with buffer standards of pH 4, pH 7, and pH 10 prior to use. 50 ml of each yogurt drink was placed in a beaker, the probe of the pH meter was inserted and pH value was recorded. This measurement was done on opening of the yogurt. The probe was rinsed thoroughly with distilled water before being used on sample.

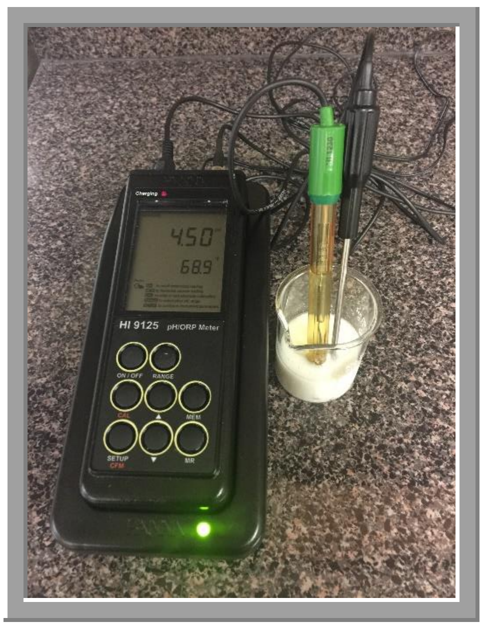

Figure 8. pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd)

3.6 Titratable acidity (TTA)

The titratable acidity was measured triplicated by the standard titratable method of AOAC (1990), and expressed as % lactic acid. A mixture of yogurt and distilled water (1:9 v/v) was titrated with 0.1 M Sodium Hydroxide (NaOH) (FisherChemical, Inc.) and 0.1% phenolphthalein (Ricca Chemical, Inc.) as the color indicator until the substance reached a Ph value 8.2. The pH was read with a pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd). The volume of NaOH required to neutralize the yogurt acid was recorded at the point when the pH value was reached and last for 30 seconds. The lactic acid percentage was calculated with this equivation:

Titratable acidity (TTA) = $\frac{\text{Titre value} \times M \times 90 \times 100}{\text{Volume of sample} \times 1000}$ **(M = Molar concentration of NAOH)**

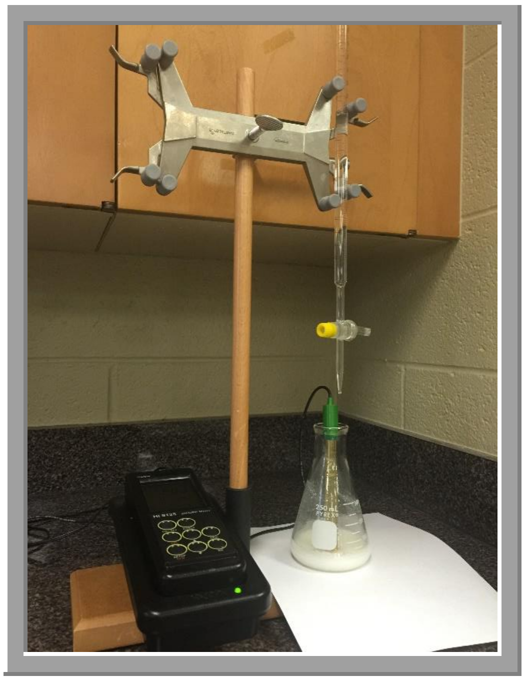

Figure 9. The titratable acidity was measured triplicated by the standard titratable method of AOAC (1990) with a pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd)

3.7 Sensory evaluation

Sensory (appearance, aroma, texture, flavor/taste, and overall preference) was evaluated by 50 untrained panelists recruited from University of Central Oklahoma. A total of 50 untrained subjects, including 37 females and 13 males between the age of 18-61 years old, volunteered to complete the sensory evaluation. Subjects conducted the evaluation individually with the privacy divider placed in the HES building in the University of Central Oklahoma, Edmond, OK. All participants were asked to sign the informed consent form and informed consent with allergy and medical release form prior to the testing. Water and crackers were provided to cleanse the palate while tasting. A range from 9 (extremely like), 5 (neither like nor dislike), to 1 (extremely dislike) was used. A control sample and sample with 1% and 2% chickpea flour were given to each panelist. About 50 ml of each sample was presented in a plastic container with the label of A, B, and C. Sample A and C were the ones containing 1% and 2% chickpea flour respectively. Sample B was plain yogurt without any chickpea flour and used as a control product. All these three samples were served in a random order (Clark, Bodyfelt, Costello, & Drake, 2009). All study procedures were approved by the Institutional Review Board (IRB) of the University of Central Oklahoma with the IRB # 15147.

Yogurt sensory evaluation sheet

Age: 15-20___ 21-25___ 26-30___ 31-35___ 36-40____ 41-45____ 46-50___

Gender: Female ____ Male ____

Major: ________

Rating scale: rate each sample on a scale from 1-9, in which 1 = “disliked extremely”, 5 = “neither liked nor disliked” and 9 = “liked extremely”.

Characteristics	Sample A	Sample B	Sample C
Appearance			
Aroma			

Texture			
Flavor/taste			
Overall preference			

3.8 Microbial counts

The bacterial counts were processed in triplicate immediately after culture inoculation or after 7, 14, 21 days storage. Every procedure was carried out in sterile conditions and on the basis of the pour plate technique (Kodaka, Mizuochi, Teramura, & Nirazuka, 2005). Lactobacillus delbrueckii subsp. Bulgaricus were counted in MRS agar (bioWORLD, Inc.) with pH adjusted to 5.2 by anaerobic incubation at 37°C for 72 h. *Streptococus thermophilus* were enumerated on M17 agar (Becton, Dickinson and Company) by aerobic incubation at 45°C for 72 h (Ashraf & Shah, 2011) (R. Dave & Shah, 1996).

The procedures of plating methodology followed the guideline of International Standardization Organization (ISO) (2003). 1.0 ml TWEEN®-80 (Sigma-Aldrich, Inc.) was added in every 1 Liter MRS agar mixture. 1.0 M hydrochloric acid (HCl) was used to adjust the pH of MRS agar to 5.2. 10% w/v of sterile lactose (FisherChemical, Inc.) was added into M17 agar. Subsequently, separate sterile pipets, prepare appropriate decimal dilutions were used for each sample. The contents were mixed thoroughly by repeated shaking. 0.1 ml of each dilution was pipetted into separate, duplicate, appropriately marked plates The samples were spread with sterile spreaders. The agar was solidified and solidified petri dishes were inverted. MRS plates were incubated in an anaerobic chamber (FisherScientific, Inc.) for 72 hrs at 37°C. M17 plates were incubated for 72 hrs at 45°C. After incubation, the formed colonies were enumerated and the results were expressed as CFU/ ml. The selectivity of the viability was confirmed by the Gram Stain method with a microscopic examination.

Figure 10. Anaerobic chamber (FisherScientific, Inc.)

3.9 Viscosity

The viscosity was measured using a digital rotary viscometer (Model NDJ-9S) with a spindle No. 3 at 30 rpm for 40 seconds. The samples were at the temperature of 21°C. Operation procedures were done following the operation manual. The test was replicated three times.

Figure 11. Digital rotary viscometer (Model NDJ-9S)

3.10 Color

Color measurement was performed with a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV, 2.54-cm diameter aperture, illuminant A, 10° observer), measurements were conducted triplicated at D65/10* (standard daylight; CIE, 1986) at room temperature.

Yogurt samples were put in a beaker with the outside diameter of 90mm, wrapped with aluminum film. Each sample contained 95mm depth of yogurt. Before measuring, stirred samples and flattened the surface. Results were recorded as CIE lab color parameters: L*(lightness), a*(red/greenness), and b*(yellow/blueness).

3.11 Statistical Analysis

All tests were conducted in triplicate. An analysis of variance (ANOVA) were performed using general linear models procedure to identify significant differences ($p<0.05$) among the samples, followed by Tukey’s test. All statistical analyses were carried out using SPSS (SPSS 20.0, IBM Crop, Armonk, NY).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Effects of chickpea flour on pH and titratable acidity

Table 2. pH* of yogurt supplementation with chickpea flour during 21-day storage period at 4°C.

Treatment**	pH			
	Day 1	Day 7	Day 14	Day 21
Control	4.21±0.01Aa	4.10±0.00Ab	4.03±0.01Ac	3.91±0.01Ad
1%	4.12±0.01Ba	4.01±0.01Bb	3.94±0.01Bc	3.82±0.01Bd
2%	4.11±0.01BCa	4.02±0.02Bb	3.95±0.01Bc	3.84±0.02Bd
3%	4.10±0.02BCa	4.00±0.00Bb	3.96±0.01Bc	3.84±0.01Bd
5%	4.08±0.00Ca	3.99±0.01Bb	3.92±0.01Bc	3.80±0.01Bd

*Means ± standard deviations of 3 measurements.

**Control = starter culture only; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation.
Means followed by the same letter (ABC) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test ($p<0.05$).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test ($p<0.05$).

Table 3. Titratable acidity* of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

Treatment**	Titratable acidity (% lactic acid)			
	Day 1	Day 7	Day 14	Day 21

Control	0.783±0.01Aa	0.837±0.01Ab	0.855±0.01Ab	0.906±0.01Ac
1%	0.876±0.01Ba	0.921±0.01Bb	0.945±0.00Bb	1.014±0.02Bc
2%	0.8355±0.03Ba	0.969±0.01Bb	0.945±0.01Bb	1.047±0.03BCc
3%	0.933±0.01Ca	1.029±0.03Cb	1.005±0.02Cb	1.104±0.02Cc
5%	0.948±0.00Ca	1.047±0.01Cb	1.119±0.01Dc	1.182±0.01Dd

*Means ± standard deviations of 3 measurements.
**Control = starter culture only; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation.
Means followed by the same letter (ABCD) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test ($p<0.05$).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test ($p<0.05$).

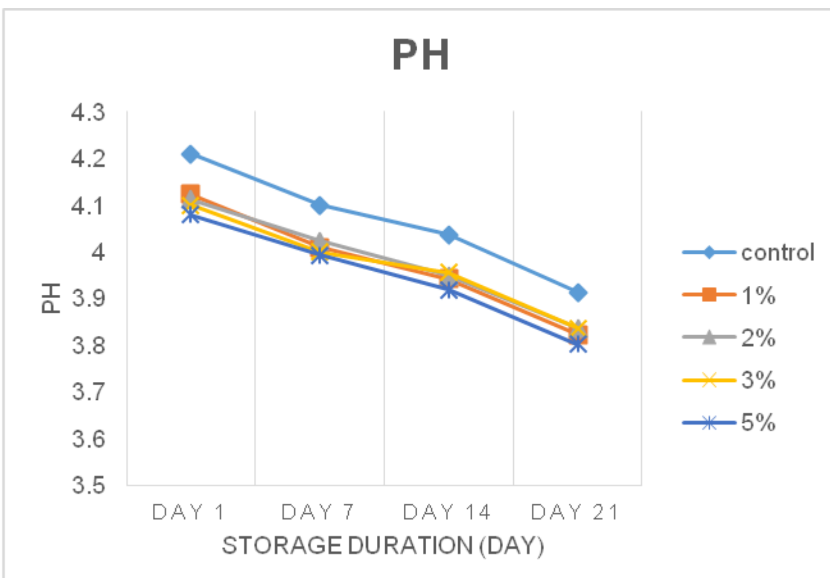

Figure 12. pH values of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

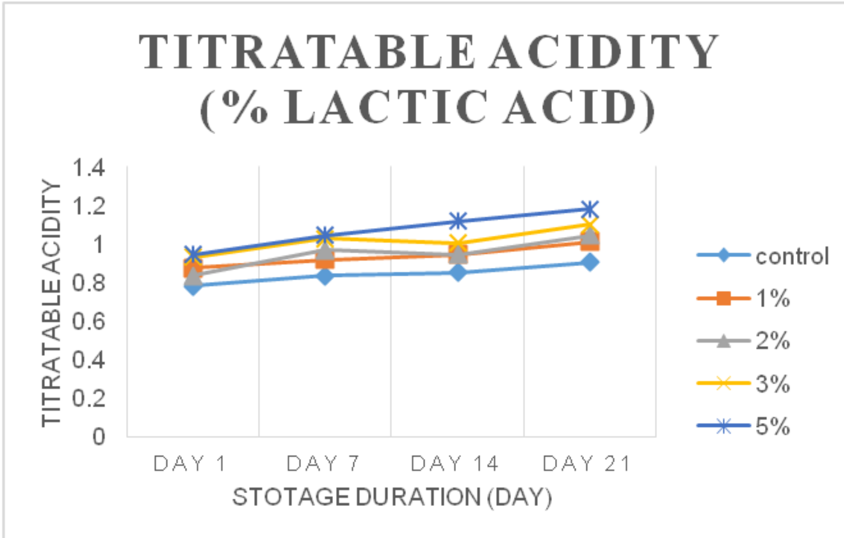

Figure 13. Titratable acidity values of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

Both the maximum pH and minimum titratable acidity value express the acidity of yogurt appropriately. Though the titratable acidity were recommended by FDA, the pH can be an even more practical, accurate, convenient measuring method than titratable acidity, especially when there is an addition of color interfering ingredients. In accordance with FDA (21 C.F.R. § 131.200 2015) definition, the titratable acidity of yogurt should be no less than 0.7 percent expressed as lactic acid or the pH for yogurt should be 4.6 or lower, prior to the addition of optional ingredients. FDA has recognized that 0.9 percent titratable acidity is too tart for some consumers and 7.5 percent is more conforming with industry practice. Recently, the IFDA urged the FDA to modify the standard requirement of titratable acidity level, which is no less than 0.6 percent in the "white mass portion" (IFDA, 2015).

In section 1, the pH values of the yogurt samples are summarized in Table 2. As seen in the graph, the lowest mean pH was found at the 21st day with 5% chickpea flour. In this study, the pH values of all treatments ranged from 3.80 to 4.21 (Table 2.). The pH value of control samples was significantly higher than that of other treatment samples. The mean pH changes differently over time depending on the concentration of chickpea flour.

During the 21-day period storage at 4°C, there were declines in the pH value of each group. For the samples with 2% and 3% of chickpea flour, the pH level was not statistically significantly different between 0 day and 7 day tests. In addition, there was no significant difference between the pH level of control samples between 7 day and 14 day. The pH level of the other sample was statistically significantly reduced during 21-day storage period.

In section 1, the titratable acidity (TTA) is shown in Table 3. The overall TTA of all samples generally increased across the 21-day period storage time. The TTA of the yogurt was significantly higher in chickpea flour containing yogurts than yogurts without any chickpea flour supplement. Compared to the other samples, the TTA value of yogurt supplemented with 5% chickpea flour was the highest.

The acidity of yogurt can be affected by the metabolic activity of the organisms in yogurt during the storage time. A high acidity value indicates a high activity of microorganisms. Although the pH of yogurt was defined as 4.6 or lower by FDA (21 C.F.R. § 131.200 2015), the pH of the final product mainly depends on the preferences of customers. Customers may accept a yogurt with the pH of 4.2, even a yogurt with the pH of 3.7-3.8, such as Greek style yogurt that contains a high total solid and fat (Robinson & Itsaranuwat, 2008).

According to the observation of Zare (Zare et al., 2012), some pulse ingredients, such as pea fiber, lentil flour, pea protein, and chickpea flour have the ability to increase the acidity value of yogurt with probiotic bacteria. Some similar research focused on fortifying low-fat yogurt with inulin, however, observed a decline of pH and an enhancement of titratable acidity during the 14-day storage period at 4° (Özer, Akin, & Özer, 2005). The similar changes in pH and TTA during a 7-day storage period were also found in the study by De Souza Oliveira (De

Souza Oliveira, Perego, Converti, & De Oliveira, 2009). They also found that different amounts of inulin addition had different influence on the production of lactic acid of yogurt containing different probiotic strains. The decrease of pH value and increase of TTA value influenced by chickpea addition also confirmed by the results of Bakr (Bakr, 2013), who reported the presence of chickpea water extract had effects on the decrease on the pH and an increase on the TTA during a 21-day storage period at 4℃.

4.2 Effects of chickpea flour on viscosity

Table 4. Viscosity* of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

Treatment**	Viscosity Pa*s			
	Day 1	Day 7	Day 14	Day 21
Control	2.278±0.364Aa	2.233±0.307Aa	2.044±0.671Aa	1.889±0.109Aa
1%	2.608±0.345Aa	2.808±0.269Aa	2.789±0.700Aa	2.269±0.113Aa
2%	2.901±0.549Aa	3.190±0.525Aa	2.974±0.344Aab	2.221±0.162Ab
3%	2.234±480.339Aa	3.165±0.288Aa	2.509±0.306Aab	2.097±0.078Ab
5%	2.380±62.482Aa	3.078±0.260Aa	2.021±0.270Ab	1.949±0.209Ab

*Means ± standard deviations of 3 measurements.
**Control = starter culture only; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation.
Means followed by the same letter (ABC) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test ($p<0.05$).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test ($p<0.05$).

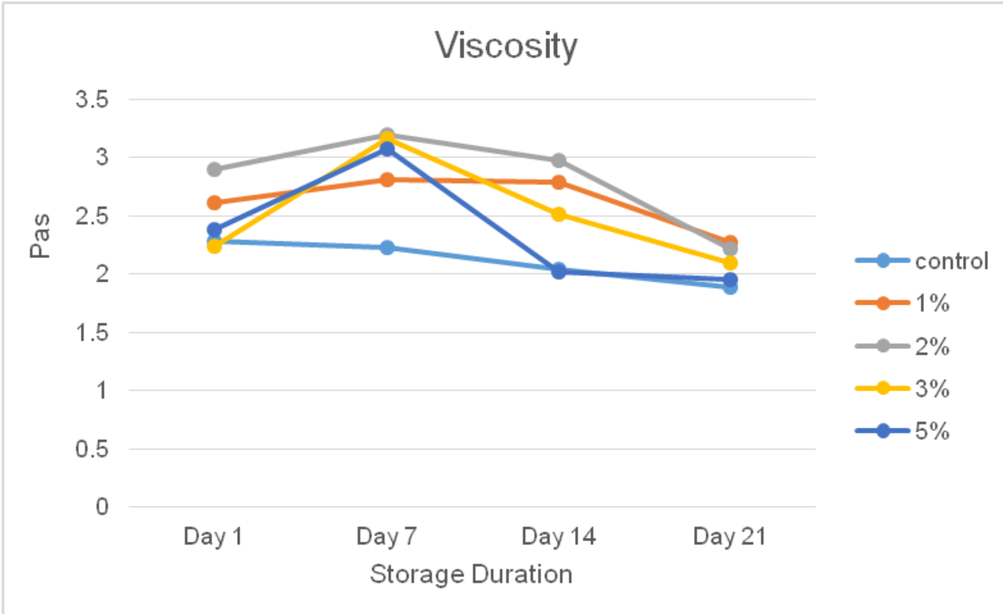

Figure 14. Viscosity of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

The estimated value of viscosity (Table 4.) indicated that the addition of chickpea flour did not influence the viscosity ($p<0.05$) at the same point of storage. Besides, no significant differences ($p>0.05$) were observed in the viscosity measurements with control sample and sample with 1% of chickpea flour across the whole storage period. Nevertheless, there are significant decreases ($p<0.05$) between 7day and 21day point in samples with 2% and 3% chickpea flour. A significant ($p<0.05$) drop of viscosity value was recorded at 7day, 14day, and 21day points in samples with 5% chickpea. These results did not accord to the earlier studies (Cruz et al., 2013), who reported a higher apparent viscosity of yogurt with supplement with oligofructose at the 1 day of storage and a greater viscosity of yogurt without supplement with oligofructose at the 28 day of storage. The inconsistent behavior of viscosity among 2%, 3% and 5% fortified samples during storage can be related to a high activity of microorganisms, which had an effect on protein network interaction and then viscosity (Debon, Prudêncio, & Petrus, 2010). Moreover, this disaccord with other studies of yogurt with prebiotic, such as inulin (Kip, Meyer, & Jellema, 2006) can be explained as chickpea flour does not have the ability to retain water as inulin has, which can promote a strong gel behavior (Meyer, Bayarri, Tárrega, & Costell, 2011).

These results demonstrated that the addition of chickpea flour had no influence in the viscosity of yogurt.

4.3 Effects of chickpea flour on microbial growth

Section 1

Table 5. *L. delbrueckii subsp. Bulgaricus* (LB) counts* of yogurt supplementation with chickpea flour fermented at 42° C for 6 hours in 21-day storage period at 4°C (fermented at 42°C for 6 hours).

Treatment**	Log CFU/mL***			
	Day1	Day 7	Day 14	Day 21
Control	7.99±0.25Aa	7.56±0.45Aa	6.36±0.32Ab	5.87±0.81Ab
1%	8.22±0.04Aa	7.98±0.33Aab	7.24±0.23Bc	6.88±0.37ABbc
2%	8.66±0.04Ba	8.09±0.16Aab	7.82±0.21BCb	7.47±0.37Bb
3%	8.71±0.05Ba	8.24±0.15Aab	8.04±0.10Cb	7.74±0.27Bb
5%	8.85±0.04Ba	8.33±0.16Aab	8.06±0.25Cb	7.76±0.29Bb

*Means ± standard deviations of 3 measurements.
**Control = starter culture only; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation.
***Log colony forming units/ml
Means followed by the same letter (ABC) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test ($p<0.05$).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test ($p<0.05$).

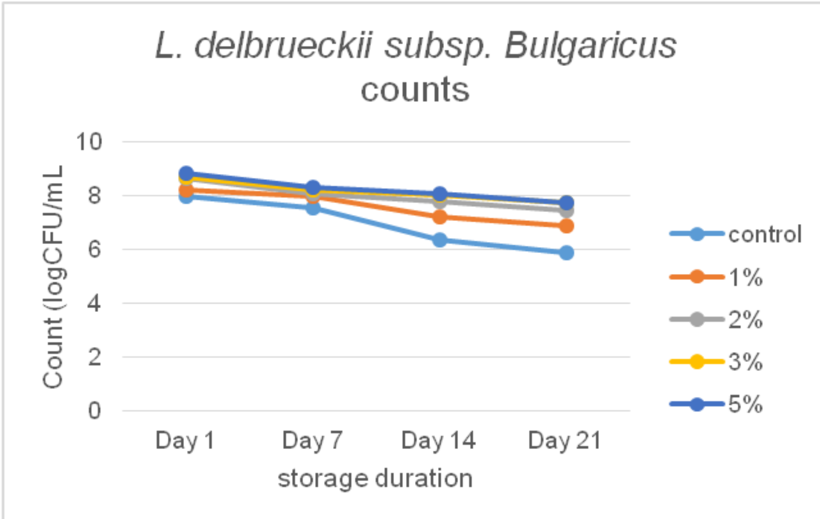

Figure 15. *L. delbrueckii subsp. Bulgaricus* counts of yogurt supplementation with chickpea flour fermented at 42° C for 6 hours in 21-day storage period at 4°C (fermented at 42° C for 6 hours).

According to the definition based on FDA (21 C.F.R. § 131.200 2015), the minimum level of live and active cultures should be 7 Log CFU/ml after production and ideally contained an expectation of 6 Log CFU/ml throughout the shelf life. Customers now have the aspirations of obtaining healthy benefits from the live and active cultures in yogurt (Hill et al., 2014).

Compared to the control through the overall storage period, the growth of LB in yogurt supplemented with chickpea flour was significantly greater ($p<0.05$) (Table 5.). The number of probiotic bacteria (*L. delbrueckii subsp. Bulgaricus*) ranged from 7.99 log CFU /ml (control) to 8.85 log CFU /ml (5%) with significant difference ($p<0.05$) between the chickpea flour enriched samples and control sample at the initial day. During the 21-day period, the viability of the probiotic bacteria (LB) slightly reduced among all the samples supplemented with and without chickpea flour. However, the sample with 5% chickpea flour maintained a stable microbial count of 7.76 log CFU /ml at day 21. These results can be attributed to the functional capability of prebiotic in stimulating the growth of probiotics, which were also confirmed by Bakr (Bakr,

2013) who used chickpea water extract in yogurt fermentation.

These results were inconsistent with another study that observed no significant effect of the addition of oligofructose on the enumeration of *S. thermophilies* and *L. bulgaricus* (Cruz et al., 2013).

Section 2

Table 6. *L. delbrueckii subsp. Bulgaricus* (LB) counts* of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

Treatment**	Log CFU/mL***			
	Day1	Day 7	Day 14	Day 21
4.5 hrs Control	5.68±0.34Aa	7.42±0.14Ab	7.25±0.10Ab	7.11±0.24Ab
4.5 hrs 1%	6.30±0.03Ba	7.77±0.09Bb	7.38±0.17Ac	7.19±0.12Abc
4.5 hrs 2%	6.17±0.08Ba	7.86±0.05Bb	7.59±0.16Bb	7.47±0.37Ab
6 hrs control	6.21±0.10Ba	7.59±0.07Ab	7.26±0.19Ab	6.94±0.48Ab

*Means ± standard deviations of 3 measurements.
**4.5 hrs Control = starter culture only, fermented for 4.5 hours; 4.5 hrs 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented for 4.5 hours. 4.5 hrs 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented for 4.5 hours. 6 hrs Control = starter culture only, fermented for 6 hours
***Log colony forming units/ml
Means followed by the same letter (ABC) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test ($p<0.05$).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test ($p<0.05$).

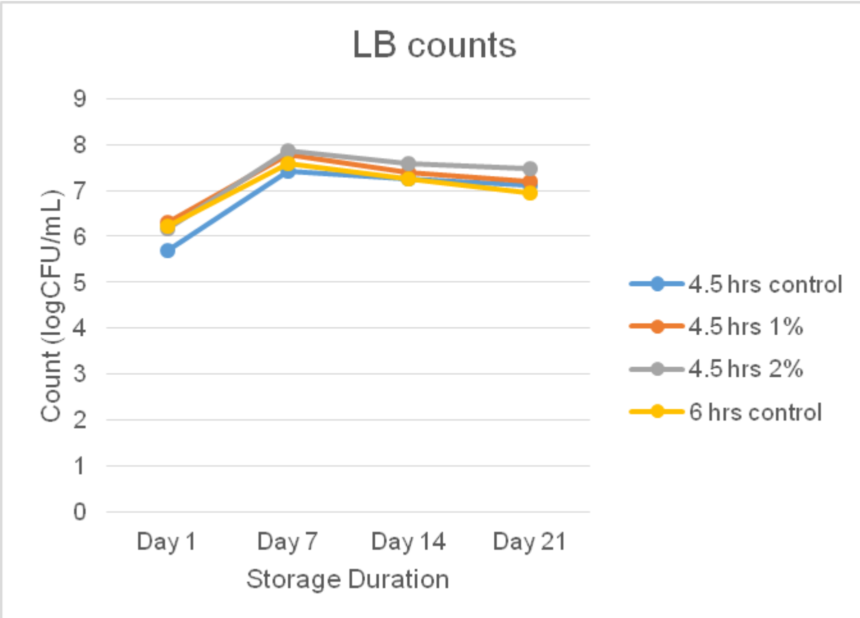

Figure 16. *L. delbrueckii subsp. Bulgaricus* (LB) counts of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

Table 7 *S. thermophiles* (ST) counts* of yogurt supplementation with chickpea flour in 21-day storage period at 4°C. (fermented at 42°C until pH reached 4.5±0.05).

Treatment**	Log CFU/mL***			
	Day1	Day 7	Day 14	Day 21
4.5 hrs Control	6.44±0.11Aa	6.67±0.18Aa	6.50±0.12Aa	6.69±0.11Aa
4.5 hrs 1%	7.60±0.11Ba	7.17±0.12Bab	7.16±0.02Bb	7.57±0.40Bab
4.5 hrs 2%	7.57±0.24Ba	7.13±0.11Bab	7.16±0.05Bb	7.46±0.32Bab
6 hrs control	6.17±0.13Aa	6.56±0.33Aa	6.18±0.03Ca	6.44±0.17Aa

*Means ± standard deviations of 3 measurements.
**4.5 hrs Control = starter culture only, fermented for 4.5 hours; 4.5 hrs 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented for 4.5 hours. 4.5 hrs 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented for 4.5 hours. 6 hrs Control = starter culture only, fermented for 6 hours
***Log colony forming units/ml
Means followed by the same letter (ABC) in the same column are not significantly different between each concentration of chickpea flour on the same storage day, according to Tukey test

(p<0.05).
Means followed by the same letter (abcd) in the same row are not significantly different for a particular day of storage for each parameter, according to Tukey test (p<0.05).

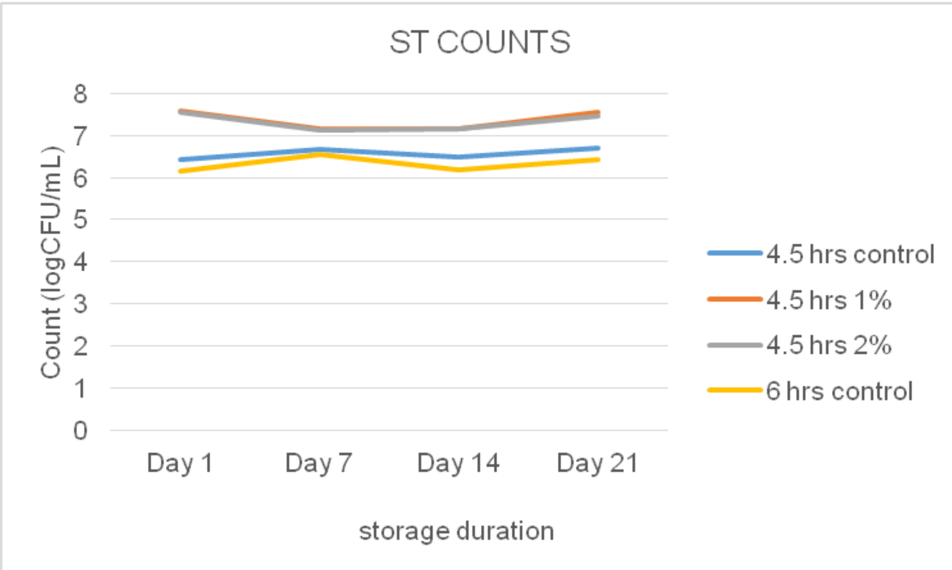

Figure 17. *S. thermophiles* (ST) counts of yogurt supplementation with chickpea flour in 21-day storage period at 4°C.

In section 2, both the LB strain and ST strain counts were significantly (p<0.05) greater than the control samples (Table6. Table7.). There was no significant difference in the LB cell counts between the chickpea fortifying samples and 6 hrs control sample (Table 6.). Nonetheless, the LB bacteria growth of sample with 2% chickpea flour, fermented 4.5 hours, significantly increased from 6.17 to 7.59 log CFU/ml after 14 days of cold storage (Table 6.). In addition, the ST bacteria counts were almost constant and did not have a significant difference (p>0.05) in every sample through the 21-day storage period (Table 7.).

Similarly, Agil et al. (Agil et al., 2013) observed that lentils had a selective positive effect on growth of probiotic bacteria. This is accordance with another study (Zare, Boye, Orsat, Champagne, & Simpson, 2011) indicated that lentil flour can promote the growth of *L. bulgaricus*, but not *S. thermophiles*. Another research that compared the effects of different pulses ingredients observed that lentil flour had the most positive effect on the growth of lactobacilli, and pulse and soy ingredients strongly promoted benefits on the growth of two probiotic bacteria, *Lactobacilus rhamuosus* AD200 and *Lactobacilus acidophilus* AD200 (Zare et al., 2012).

4.4 Effects of chickpea flour on sensory evaluation

Table 8. Sensory properties* of yogurt supplementation with chickpea flour at 4°C, immediately after production. (fermented at 42°C until pH reached 4.5±0.05).

Treatment **	Appearance	Aroma	Texture	Flavor/taste	Overall preference
	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD
Control	5.08±2.28A	5.56±2.48A	5.14±2.53A	4.5±2.84A	4.92±2.63A
1%	5.48±2.42A	5.44±2.33A	4.82±2.65A	3.52±2.66A	4.3±2.75A
2%	4.68±2.25A	5.34±2.45A	5.02±2.42A	4.02±2.54A	4.54±2.38A

*Means ± standard deviations of 3 measurements.
**Control = starter culture only, fermented until pH reached 4.5±0.05; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented until pH reached 4.5±0.05; 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented until pH reached 4.5±0.05.
Means followed by the same letter (ABC) in the same row are not significantly different for each concentration of chickpea flour according to Tukey test ($p<0.05$).

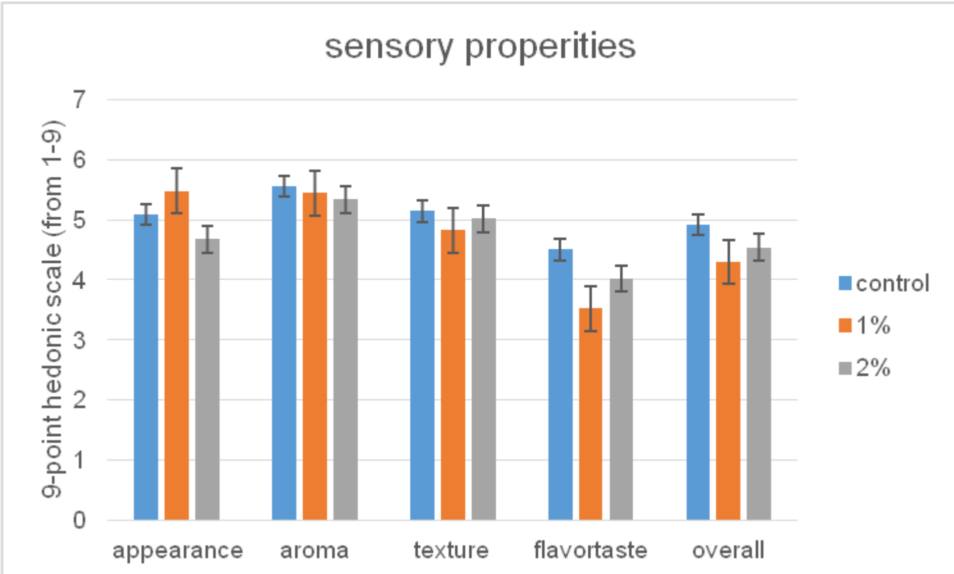

Figure 18. Sensory properties of yogurt supplementation with chickpea flour at 4°C, immediately after production.

Besides the other analyses of yogurt, sensory evaluation of dairy products is an invaluable tool that can impel the development of dairy industry in order to provide a lasting high quality for customers (Clark et al., 2009).

The results of the sensory evaluation revealed that there was no significant influence ($P>0.05$) in the appearance, aroma, texture, flavor/taste, and overall preference level among the control sample, and samples with 1% and 2% of chickpea flour (Table 8.). The sample with 1% chickpea flour had the highest appearance score (Table 8.). A similar study reported that yogurt enriched with chickpea water extract performed better in texture. In addition, there was a reduction on the score of the sweetness, color, and overall preference with respect to samples containing chickpea water extract (Bakr, 2013).

4.5 Effects of chickpea flour on color

Table 9. Color* of yogurt supplementation with chickpea flour at 4°C, immediately after production. (fermented at 42°C until pH reached 4.5±0.05).

Treatment**	Color parameter***		
	L*	a*	b*
Control	96.81±0.16A	-1.90±0.03A	10.20±0.14A
1%	96.93±0.22A	-1.77±0.16A	9.99±0.19A
2%	96.32±0.51A	-1.79±0.12A	10.68±0.12A
3%	96.84±0.13A	-1.71±0.03A	10.35±0.03A
5%	93.39±0.46A	-1.86±0.04A	10.96±0.14A

*Means ± standard deviations of 3 measurements.
**Control = starter culture only, fermented until pH reached 4.5±0.05; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented until pH reached 4.5±0.05. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented until pH reached 4.5±0.05. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation, fermented until pH reached 4.5±0.05.
*** L* = lightness; a* = redness (+) and blueness (–); b* = yellowness.
Means followed by the same letter (ABC) in the same row are not significantly different for each concentration of chickpea flour according to Tukey test ($p<0.05$).

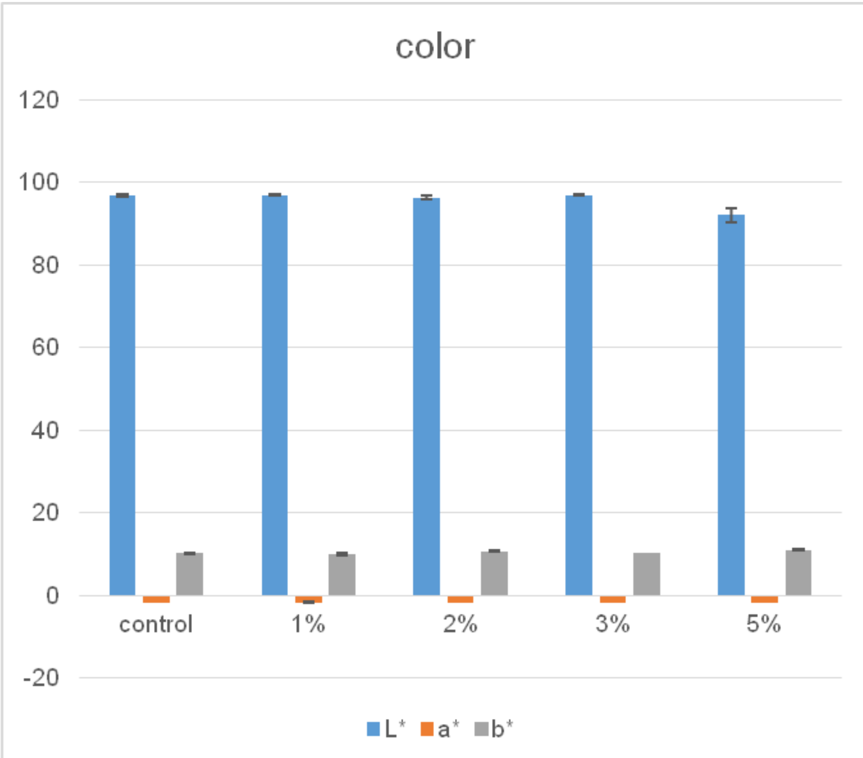

Figure 19. Color of yogurt supplemented with 1%, 2%, 3%, and 5%, chickpea flour and control sample at production day 1 at 4°C

L* = lightness; a* = redness (+) and blueness (–); b* = yellowness.

A clean white appearance with a pleasant sheen seems to most often meet the demand color of a natural set yogurt. The color of yogurt is commonly associated with the acceptance of consumers and marketability. Thus, it is important for a functional yogurt to provide both the nutrients benefits and be customer friendly. The standard instrument, such as HunterLab spectrophotometer, is more precise than the visual comparison (Robinson & Itsaranuwat, 2008).

The yogurt was of a white color immediately after production, as indicated by higher L* value, lower a* and b* value in Figure 19. Data did not show significant differences (P>0.05) among samples with different concentrations of chickpea flour and control samples. This means that the addition of chickpea flour from 1% to 5% did not change the color of yogurt.

4.6 Effects of chickpea flour on fermentation time

Table 10. pH* of yogurt supplementation with chickpea flour in different fermentation time, fermented at 42ºC until pH reached 4.5±0.05

Treatment **	pH***						
	3hrs	3.5hrs	4hrs	4.5hrs	5hrs	5.5hrs	6hrs
Control	6.16±0.006A	5.78±0.012A	5.23±0.006A	5.02±0.020A	4.83±0.006	4.66±0.006	4.53±0.026
1%	5.58±0.006B	5.26±0.006B	4.95±0.010B	4.58±0.012BC			
2%	5.27±0.012C	5.05±0.006C	4.86±0.010C	4.59±0.010B			
3%	5.18±0.012D	5.05±0.012C	4.72±0.015D	4.54±0.012C			
5%	5.16±0.015D	5.03±0.015C	4.74±0.012D	4.52±0.017C			

*Means ± standard deviations of 3 measurements.
**Control = starter culture only; 1% = addition of 1.0 g chickpea flour per 100 ml of milk prior to fermentation. 2% = addition of 2.0 g chickpea flour per 100 ml of milk prior to fermentation. 3% = addition of 3.0 g chickpea flour per 100 ml of milk prior to fermentation. 5% = addition of 5.0 g chickpea flour per 100 ml of milk prior to fermentation.
***pH of yogurt fermented at 42°C until pH reached 4.5±0.05
Means followed by the same letter (ABCD) in the same row are not significantly different for each concentration of chickpea flour according to Tukey test ($p<0.05$).

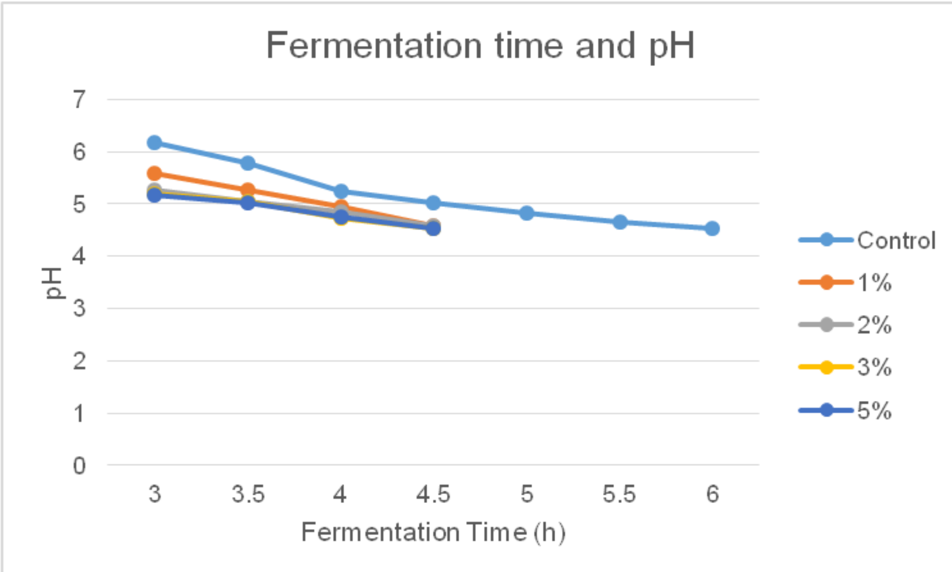

Figure 20. pH of yogurt supplementation with chickpea flour in different fermentation time, fermented at 42ºC until pH reached 4.5±0.05

The average fermentation time for all samples reached the pH 4.5±0.05 at 42°C is shown in Table 10. and Figure20. It is of interest to observe that all samples reached the target pH at 4.5 hours except the control one. The shortest time observed (4.5h) was for samples with 1%, 2%, 3%, and 5% of chickpea flour. The time to reach the target pH was 6 hours in control samples and 4.5 hours in chickpea supplemented samples, and there were significant differences ($p<0.05$) between them. In addition, the final pH of all the samples averaged 4.5±0.05 without significant difference ($p<0.05$). A similar view was reported by Zare et al. (Zare et al., 2012) where the adding of pulse ingredients, such as pea fiber, lentil flour, soy flour, soy protein, and chickpea flour, had reduced the pH significantly after 4 hour fermentation. Though there is a lack of research on the specific pulses component that contributes to the reduction of the fermentation time, it is most likely that a high content of dietary fiber in pulses contributed to this. These dietary fibers enhanced the production of lactose hydrolytic enzyme due to the capability of promoting activity of organisms in yogurt. Therefore, there is faster decrease in pH with fermentation.

CHAPTER FIVE: CONCLUSION AND FUTURE DIRECTIONS

In this study, achieved data from the pH and TTA value indicated that the inclusion of chickpea flour significantly enhanced the acidification of yogurt, as a consequence, reduced the fermentation time from 6 hours to 4.5 hours for reaching the final pH. The lowest pH value and the highest TTA value were recorded after 21 days refrigerated storage in yogurt with 5% of chickpea flour.

The viscosity value showed a slight decrease during 21-day period of refrigerated storage in samples with and without chickpea flours. No significant difference was observed among different concentration of chickpea flour and control sample at any point of storage day. The presence of chickpea flour did not affect the color and sensory evaluation compared with the control sample (plain yogurt).

Furthermore, the addition of chickpea flour in yogurt that was fermented for the same time resulted in significantly higher *L. delbrueckii subsp. Bulgaricus* cell counts during the 21-day storage period. Counts of *L. delbrueckii subsp. Bulgaricus* in Samples supplemented with 5% chickpea flour still remained significant greater after 21 days of storage at 4°C. Besides, chickpea flour enhanced the number of *S. thermophiles* across the whole storage period on both pH dependent and fermentation time dependent conditions.

In conclusion, chickpea flour has the potential of providing more nutritional benefits and high quality in fermented dairy products on the basis of physico-chemical, microbial, color, viscosity, and sensory evaluation. It can be utilized as a natural supplement to fortify yogurt.

In the future, studies with specific component extracted from chickpeas are necessary to explore more health-related potentials of chickpea in fermented dairy products.

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