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Yogurt cultured milk powder as a substitute for yogurt powder

Lijie Song

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YOGURT CULTURED MILK POWDER AS A SUBSTITUTE FOR YOGURT POWDER

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Animal Sciences

by
Lijie Song
B.S., Jimei University, 2011
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ABSTRACT

Commercial yogurt powder has to go through drying process which kills the yogurt culture, so the health benefit of the yogurt culture bacteria are lost. Also, upon reconstitution commercial yogurt powder does not taste like yogurt, it is sour and off flavored. The hypothesis of this study was that yogurt cultured milk powder would have better culture bacterial counts, better physico-chemical and sensory characteristics than commercial yogurt powder currently available. Commercial yogurt powder (CYP) was the control and yogurt cultured milk powder (YCMP) was the treatment. Freeze-dried yogurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* at ratio 1:1) was added to milk powder at 10^7 cfu/g upon reconstitution. Microbial and physico-chemical characteristics of the reconstituted CYP and YCMP were analyzed daily for the first week and then weekly for a period of 8 weeks (at 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 days) after reconstitution. Three replications of each treatment were conducted. Sensory consumer testing of CYP and YCMP upon reconstitution was conducted with 100 panelists. Data were analyzed by Proc GLM of Statistical Analysis System. YCMP had 5 log cfu/ml higher counts of *Streptococcus thermophilus* compared to the control (CYP) at 56 days. Also, *Lactobacillus bulgaricus* counts of YCMP at 28 days was 6.55 log cfu/ml and at 56 days was 5.35 log cfu/ml while the CYP at 28 days onwards had no counts. YCMP had significantly higher apparent viscosity, pH, L*, appearance, sensory color, aroma, taste, thickness, overall liking, consumer acceptability and purchase intent compared to CYP.

CHAPTER 1: INTRODUCTION

1.1 YOGURTS

Yogurt is a very important dairy product over the world in recent times. According to the Code of Federal Regulations of the FDA (CFR, 2013): Yogurt is 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 thermophilus*. This milk product obtained by the fermentation of milk by the action of symbiotic cultures and resulting in reduction of pH with coagulation. Yogurt, before adding bulky flavors, contains not less than 3.25 percent milk fat and not less than 8.25 percent milk solids not fat, and has a titratable acidity of not less than 0.9 percent, expressed as lactic acid. The food may be homogenized and shall be pasteurized or ultra-pasteurized prior to the addition of the bacterial culture. Flavoring ingredients may be added after pasteurization or ultra-pasteurization. To extend the shelf life of the food, yogurt may be heat treated after culturing is completed, to destroy viable microorganisms (CFR, 2013). Yogurt have now become a popular product for researchers worldwide as it has been claimed to be a healthy food. Over the past few decades, the development of yogurt as a product that has health benefits for consumers has included the addition of probiotic microorganisms (i.e., *Lactobacillus* spp. and *Bifidobacterium* spp.) and prebiotic ingredients can stimulate the growth of these organisms in the intestinal tract (Shah, 2001 and Tamime, 2006).

1.1.1 Market of Yogurts

The yogurt market has seen some very strong growth since 2003. Yogurt sales and consumption were increased in the last five years (Dairy facts 2009, 2010, 2011 and 2012). The percentage increase in yogurt sales and consumption was 3.6% between 2007 and 2008, 5.9% between 2008

and 2009, 8.3% between 2009 and 2010 and 1.3% between 2010 and 2011 (Dairy facts 2009, 2010, 2011 and 2012). The percent increase in yogurt sales and consumption between 2008 and 2009 was more than other dairy products (Dairy Facts 2010). According to Zenith International, yogurt is expected to rise from 11million tons in 2003 to 16 million tons in 2012 across more than 70 countries worldwide, which is equivalent to a 38% rise in consumption (Weston, 2010). Yogurt market was worth \$9.7 billion in 2005 and \$15.4 billion in 2010 (Heller, 2006).

1.1.2 Health Benefits of Yogurts

As a healthy food, the health effects of yogurt are divided into two groups: nutritional function and physiological function. The nutritional attribute is expressed as the function of supplying nutrition sufficiently, such as the source of lactose, proteins, vitamin (riboflavin, vitamin B6 and vitamin B12) and calcium. The physiological function refers to prophylactic and therapeutic functions beyond nutritional function, like antimicrobial activity, gastrointestinal infections, anticancer effects reduction in serum cholesterol and immune system stimulation (Shah, 2006 and Ashraf and Shah, 2011).

During the past decades, full fat yogurt consumption has decreased due to changes in dietary habits of consumers. Therefore, many modifications in yogurt products have been developed by manufacturer to reduce milk fat content in yogurt (Trachoo, 2002). So far, there are various nonfat and low fat yogurt available in the market.

1.2 PROBIOTICS

Probiotics play an important role in human nutrition. Probiotics are defined as “living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent

basic nutrition” (Guarner and Schaafsma, 1998). A similar definition was proposed by a United Nations and World Health Organization Expert Panel: “live micro-organisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002).

Many studies have shown that probiotics can stimulate the immune system, decrease serum cholesterol, alleviate lactose intolerance, decrease diarrheal incidence, avoid allergy, control infections and protect against cancer (O’Bryan *et. al.* 2013). Micro-organisms commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria (*Lactobacillus*, *Enterococcus*, *Streptococcus*) and to the genus *Bifidobacterium* (FAO/WHO, 2001). Foods containing such bacteria are in the category of functional foods, which are foods that have a potentially positive effect on health by adding new ingredients or more of existing ingredients. According to Soccol *et. al.*(2010), from 2007 to 2008, the global probiotic ingredients, supplements and foods market was increased from \$14.9 billion to \$15.9 billion and it was expected to increase to 19.6 billion in 2013, representing an annual growth rate of 4.3%.

Probiotics and prebiotics play an important role in dairy products. This kind of products, like yogurts and other fermented milks, fermented with lactic acid bacteria, such as *Bifidobacterium* and *Lactobacillus* strains, sugar fortified with FOS (fructo-oligosaccharides) or inulin, or food supplements containing probiotic bacteria have been available on the food market for more than 10 years (Saad *et.al.*, 2013). Figueroa *et.al.* (2011) reported yogurt and other fermented milks as leader products of functional foods comprising approximately 65% of the world functional food market.

Generally, yogurt bacteria are now characterized as lactic acid bacteria belong to the *Lactobacillaceae* and *Streptococcaceae* genera (Tamime and Deeth, 1980).). In some countries the statutory regulations may stipulate that there be a ratio of 1:1 between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, a minimum number of cfu ml⁻¹ in the final product and a pH level < 4.4 (Tamime, 2000). According to criteria of The National Yogurt Association, McLean, VA, the total population of active culture in refrigerated cup yogurt must be at least 10⁸ cfu/g at the time of manufacture. Under proper distribution and handling practices, the total numbers in the active culture yogurt at the time of consumption will be a minimum of 10⁷ cfu/g (Chandan, 1999). To confer health benefits, probiotic bacteria should be viable at the time of consumption at a recommended concentration of 6-8 log cfu/g (Ross et. al., 2005, Vasiljevic and Shah, 2008)

1.2.1 *Lactobacillus delbrueckii* subsp. *bulgaricus*

Lactobacillus delbrueckii subsp. *bulgaricus* (*L. bulgaricus*), as probiotic culture, belongs to the acidophilus group of lactobacilli which includes *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus gasseri* (van de Guchte et. al., 2006). It is gram positive, facultative anaerobe and homofermentative lactic acid bacteria (Delley and Germond, 2002). *L. bulgaricus* can ferment glucose, fructose, galactose and lactose to lactic acid. It produces D (-)-lactic acid up to 1.7 % in milk and has a growth temperature between 22°C and 60°C (Rasic and Kurmann, 1978). According to Mayra- Makinen and Bigret (1993) the optimum growth temperature for it is 40°C to 50°C. In yogurt fermentation, *L. bulgaricus* is subjected to decrease environmental pH for food product preservation. Subsequently the bacterium has to survive in highly acidic gastric juice if it reaches to the small intestine in a viable state and exerts the expected beneficial effects (Henriksson et.al., 1999 and Lee and Selminen, 1995). The proteins in milk are of excellent quality biologically

and both the caseins and whey proteins (α -La and β -Lg) are well endowed with essential amino acids. One characteristic is that the proteins in yogurt are totally digestible, a feature enhanced by the fact that some degree of initial proteolysis is caused by the starter organisms themselves (Tamime and Robinson, 2000). The other pertinent characteristic is that the milk proteins in yogurt are already coagulated prior to ingestion and the “soft clot” formed in the stomach may act as a role to slow the caecal transit time of lactose, so allowing the microbial lactase to ensure that lactose-intolerant consumers do not suffer discomfort (Marteau *et al.*, 1993).

The ability of probiotics to survive in an acidic environment is important for both fermentation stability and in vivo function. Thus, acid tolerance should be considered when select potentially probiotic strains (Cui *et.al.*, 2012). According to Shah and Jelen (1990), *Lactobacillus delbrueckii ssp. bulgaricus* proved to be more acid tolerant than *Streptococcus thermophilus*. Liong and Shah (2005) also reported that the most acid tolerant strains of Lactobacillus strains are *L. acidophilus* and *L. casei*. *L. bulgaricus* is commonly used together with *Streptococcus thermophilus* that they became the preferred partners for rapid milk fermentation for the production of yogurts (Delley and Germond, 2002). There are two stages involved in yogurt fermentation. In the first stage, *L. bulgaricus* stimulates the growth of *S. thermophilus* by releasing essential amino acid from casein by proteolytic activity. Meanwhile, *L. bulgaricus* grows slowly because it is microaerophilic. At the end of the first stage, the growth of *S. thermophilus* is slowed down due to the high lactic acid concentration. When *S. thermophilus* produces enough formic acid, which stimulates growth of *L. bulgaricus*, the second stage begins. By this symbiotic action the desirable acidity of the final yogurt can be achieved (Sandine and Elliker, 1970. Rasic and Kurmann, 1978).

Some *Lactobacillus bulgaricus* have immunological effects. It has been shown to exert host-mediated antitumor activity in mice (Ebina *et. al.*, 1995). In vitro experiments have revealed the mitogenic activity of extracellular polysaccharides (EPS) produced by *L. bulgaricus* (Kitazawa *et. al.*, 1998). In a study of Kitazawa *et. al.* (2003), an immunostimulatory oligonucleotide was derived from *L. bulgaricus* NIAI B6. This strain would be a good candidate of a starter culture for the production of new functional foods as “Bio-Defense Foods” (Kitazawa *et. al.* 2003)

1.2.2 *Streptococcus thermophilus*

Streptococcus thermophilus is a thermophilic lactic acid bacterium (LAB) widely used in the manufacture of yogurt products and may be regarded as the second most important industrial dairy starter after *L. lactis* (Hols *et.al.*, 2005). It is related to *Lactococcus lactis* but is phylogenetically closer to streptococcal species of the viridans group (Delorme, 2008). It is identified as anaerobic, aerotolerant, catalase negative and gram positive, growing as linear chains of ovoid non-motile coccus with 0.7-0.9 µm in diameter and unable to grow at 10°C, at pH 9.6 or in 6.5% NaCl broth (Moschetti *et. al.*, 1998, Sheman, 1937 and Harnett *et.al.*, 2011). According to Rasic and Kurmann (1978), the growth temperature for *S. thermophilus* ranges from 20°C to 50°C with an optimum of 40°C to 45°C. It can ferment glucose, fructose, lactose and saccharose to L (+)-lactic acid up to 0.7 to 0.8% in milk. Some strains of *S. thermophilus* produce neutral exopolysaccharides (EPS) which are thought to play roles in protection against detrimental environmental conditions, in cell recognition, and in biofilm formation (Broadbent *et.al.*, 2003). EPS produced by ropy *S. thermophilus* strains reduces firmness and improves viscosity, water retention and the mouth feel of yogurt (Robitaille *et.al.*, 2009).

As for health benefits, some strains of *S. thermophilus* produce bacteriocin. Some studies have characterized several bacteriocins (e.g., thermophilin 110, thermophilin 1277) produced by *S. thermophilus* that are active against *Pediococcus acidilactici*, *Clostridium butylicum*, *Clostridium sprogenes*, *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes* (Gilbreth and Somkuti, 2005, Kabuki *et. al.*, 2009). In addition, combinations of probiotic products containing *S. thermophilus* have been described to improve gastrointestinal function, such as prevention of rotaviral diarrhea in infant and reduction of both the incidence and severity of Necrotizing Enterocolitis in very low birth weight neonates (Saavedra *et. al.*, 1994 and Bin-Nun *et.al.*, 2005). Besides, according to Carper (1998), Yogurt containing *S. thermophilus* and *L. bulgaricus* was showed to decrease the incidence of lung cancer in mice.

1.2.3 *Lactobacillus acidophilus*

Traditional yogurt is made from symbiotic growth of starter bacteria *S. thermophilus* and *L. bulgaricus*. To improve the health benefits, the recent trend is to add *L. acidophilus* to yogurt (Ashraf and Shah, 2011). *L. acidophilus* is a homofermentative species, fermenting sugars into lactic acid and identified as Gram positive non-spore-forming rods with rounded ends that occur singly and in short chains (Gopal, 2011). *L. acidophilus* can be found naturally in the human and animal gastrointestinal tract and vagina (Steven and Ehrlich, 2011). According to Baati (2000), *L. acidophilus* grows at rather low pH values (below pH 5.0) and has an optimum growth temperature of around 37°C.

Lactobacillus acidophilus is a common probiotic species, some stains have been studied for potential health benefits. Some studies showed that *L. acidophilus* may prevent diarrhea in children and adults, especially effective in treating rotavirus in children (Allen *et.al.*, 2009 and Lee

et.al., 2001). For instance, *L. acidophilus* was taken at a minimum level of 10^9 cfu daily to prevent or treat some gastrointestinal (GI) disorders (WGO, 2008). Other health benefits associated with *L. acidophilus* include anticarcinogenic properties, reduction in blood pressure and serum cholesterol concentration, and increased resistance to infectious diseases (Ashraf and Shah, 2011). Besides, some strains of *L. acidophilus* produce bacteriocins, such as lactacin B which against *Salmonella enteritidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Vincent et.al., 1959), lactacin F which inhibit other lactobacilli as well as *Enterococcus faecalis* (Muriana and Klaenhammer, 1991), acidocin A which against closely related lactic acid bacteria and food-borne pathogens including *Listeria monocytogenes* (Kanatani, 1995), and acidocin B which against *Listeria monocytogenes*, *Clostridium sporogenees*, *Brochothrix thermosphacta*, *Lactobacillus fermentum* and *Lactobacillus delbrueckii subsp. bulgaricus*, but inactive against most other *Lactobacillus* species (Leer et.al. 1995).

There are many potential health or nutritional benefits from these lactic acid bacteria. Among these are: improved digestion of lactose, control of intestinal infections, control of some types of cancer and control of serum cholesterol levels (Gilliland, 1990)

1.3 YOGURT POWDER

The shelf life of yogurt is a short 5 weeks of refrigerated storage. In natural calamity areas or in food aid to less fortunate countries where the fresh yogurt is less likely to be available.

The objective of manufacturing dried yogurt in powder form is to improve the shelf life of the product and ease of use. To manufacture yogurt powder, the milk's first fermentation with yogurt cultures *L. bulgaricus* and *S. thermophilus*, until reaches a desirable pH (4.6) and then the yogurt

this produced is dried (Krasaekoopt and Bhatia, 2012). Traditionally, natural/plain yogurt, which is low in fat, is concentrated, shaped into flat rolls and sun dried (Kurmann *et al.* 1992). Nowadays, with the development of the technology, freeze-drying, spray-drying or microwave-drying are the main methods of drying yogurt. Dried yogurt requires less packaging, storage and distribution costs because of the reduction of bulk water, and the refrigeration is not needed (Kumar and Mishra, 2004). However, the first commercial attempts to produce yogurt powder were aimed at the “do-it-yourself” consumer market and the reconstituted yogurt lacked a high active number of starter culture bacteria, as well as the pleasant taste, firm body/texture and the attractive appearance of regular yogurt (Tamime and Robinson, 2000).

Yogurt powder can be used in a wide variety of food applications, including instant yogurt, replacement of fresh yogurt for beverage and dip. It can be also used in snacks, confections, bakery items and breakfast cereals, ice cream bars and fruit-yogurt dressing (Tamime and Robinson, 2000, Childs and Drake, 2008). There are also other types of blended dairy ingredients that have same flavor and function as yogurt powder (Krasaekoopt and Bhatia, 2012). Many additives are used to give the powder product a yogurt-like appearance and taste after rehydration. Some of these additives are sucrose, dextrose, stabilizers (i.e. starch, locust bean gum, xanthan gums, Na-alginate), sequestering agents and organic acid (Tamime and Robinson, 2000).

1.3.1 Drying of yogurt

Generally, yogurt is dried by freeze- , spray- or microwave vacuum-drying. It would be beneficial if the yogurt were concentrated before drying to increase its total solids to improve the efficiency of the drying process (Kumar and Mishra, 2004). Each drying method has its benefits and drawbacks.

Freeze-drying is freezing the yogurt and then reducing the surrounding pressure to allow the frozen water in the yogurt to sublime directly from the solid phase to the gas phase. Freeze-dried yogurt has the best flavor and is the more authentic product in comparison to those obtained using other conventional drying methods (Rybka and Kailasapathy, 1997). Rybka also described the freeze-drying of yogurt at -40°C for 48h. Another study showed that freezing temperature of -15, -25 and -40°C had no significant effects on the final contents of total protein casein, serum and non-protein nitrogen. The survival of lactic acid bacteria was 50-60% during freezing at -25 or -40°C (Radaeva *et.al.*, 1975)

Spray-drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. It is a well-known method for milk and yogurt drying because it allows preparation of stable and functional Products (Kumar and Mishra, 2004). Survival of yogurt bacteria is also affected by the outlet temperature in spray drying process. According to Bielecka and Majkowska (2000), the best survival of *L. bulgaricus* was 13.7-15.8% and *S. thermophilus* was 51.6-54.7% at outlet temperature ranges 70-75°C; the final moisture content of the dried product is 5.1-6.3%. At temperature below 60°C, wet powder was obtained while above 90°C, powder was not acceptable due to browning (Kim and Bhowmik, 1990). In addition, it has been reported that most of the aroma compounds and rheological characteristics of yogurt are lost during the spray-drying process (Kumar and Mishra, 2004). However, spray drying is an economical process for industrial production of viable microorganisms. Its application to preparations of lactic acid bacteria has recently received great interest (Peighambardoust *et. al.*, 2011).

Microwave vacuum drying is very well suited for the continuous drying of fragile and heat sensitive products without affecting quality. This drying method allows at low temperature may be a useful alternative to other methods likes freeze- and spray-drying (Kim and Bhowmik, 1990). The approximate survival ratio was 0.5 in microwave vacuum-dried yogurt below 45°C, 0.1 in the freeze-dried yogurt, and 0.05 in the spray-dried yogurt (Kim *et.al.*, 1997). Additional advantages of microwave vacuum-drying are shorter drying time and cheaper cost than freeze-drying method (Kim *et.al.*, 1997).

A high quality yogurt is one of good texture and includes effective amounts of active culture as well as protein, calcium and other useful nutrients. In addition, with the increasing demand of healthy eating, people prefer low-fat or non-fat products. A good instant yogurt is a dry product which can be stored for a long time and which can be reconstituted simply by adding water and stirring the mixture for several minutes to produce a product having a texture, taste and nutritional properties very similar to natural yogurt.

1.4 JUSTIFICATION

Commercial yogurt powder has to go through drying process which kills the yogurt culture, so the health benefit of the yogurt culture bacteria are lost. And also, upon reconstitution yogurt powder does not taste like yogurt, it is sour and off flavored.

However, yogurt powder provides longer and more stable shelf life than that of regular yogurt. Moreover, the reduced weight and bulk water of this dehydrated products decreases packaging, handling, and transportation costs. This product is very convenient for consumer to use since it can be store at ambient temperature for a long shelf life. There are many situations that sometimes

consumer does not have access to supermarkets purchase natural yogurt. It can also be shipped to natural calamity areas or for food aid to less fortunate countries. While the consumer can make yogurt at home, it is a time-consuming operation requiring some skill and also need the use of refrigerator to chill and store the yogurt. However, for an instant reconstituted yogurt, consumer just needs to add water into the product and stir to mix well when they want to consume yogurt.

The yogurt and yogurt drinks market is benefited greatly as consumers pay greater attention to healthy eating. In the food industry, it is important to produce high quality food product with a low cost. Upon reconstitution having a better quality yogurt than currently in available is desirable and would be beneficial to both food industry and consumers. Further study on the parameters of this kind of product is also valuable.

1.5 HYPOTHESIS

Whether yogurt cultured milk powder would have better culture bacterial counts, physico-chemical and sensory characteristics than commercial yogurt powder currently available.

1.6 OBJECTIVES

1. To enumerate *Streptococcus thermophilus* and *Lactobacillus bulgaricus* of reconstituted yogurt cultured milk powder and reconstituted commercial yogurt powder up to 8 weeks.
2. To enumerate E. coli/coliform bacterial and yeast and mold of reconstituted yogurt cultured milk powder and reconstituted commercial yogurt powder up to 8 weeks.
3. To elucidate the influence on the physico-chemical characteristics (pH, titratable acidity, color and apparent viscosity) of reconstituted yogurt cultured milk powder and reconstituted commercial yogurt powder up to 8 weeks.

4. To study the sensory characteristics of yogurt cultured milk powder without *L. acidophilus*, yogurt cultured milk powder with *L. acidophilus* and commercial yogurt powder upon reconstitution and to determine the consumer acceptability of the product.

CHAPTER 2: MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

This study consisted of commercial yogurt powder as the control and reconstituted yogurt cultured milk powder as the treatment. Freeze-dried yogurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* at ratio 1:1) was added to milk powder to the concentration of 10^7 cfu/g upon reconstitution. For the first, second and third objectives, microbial and physico-chemical characteristics of the reconstituted yogurt powder were analyzed daily for the first week and then weekly for a period of 8 weeks (at 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49, 56 days) after reconstitution. Three replications of each treatment were conducted. For the fourth objective. Sensory characteristics of consumer test of yogurt cultured milk powder with or without *L. acidophilus* and commercial yogurt powder upon reconstitution was conducted with 100 panelists. The experimental design for the apparent viscosity and sensory evaluation was randomized block design (RBD) with replications as blocks. The experimental design for microbial counts, pH, titratable acidity, color was repeated measurements.

2.2 YOGURT MANUFACTURE

For microbial characteristics and physico-chemical characteristics, two treatments of reconstituted yogurt powder were manufactured, one was commercial yogurt powder (CYP) and the other one was yogurt cultured milk powder (YCMP). The commercial yogurt powder was obtained from DairiConcepts, L.P. The CYP contains yogurt powder, pure water and blue berry puree. The YCMP contains non-fat dry milk, pure water, pectin, yogurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), citric acid and blue berry puree. Ingredients information and reconstituted yogurt formulations are showed in Table 1 and Table 2, respectively.

Table 1. Reconstituted yogurt powder ingredients information

NAME	DESCRIPTION	COMPANY
Commercial Yogurt Powder	Dry Nonfat Yogurt Powder, Kosher	DairiConcepts, L.P.
Non-fat Dry Milk	Nonfat Instant Dry Milk (fortified with Vitamins AandD)	Great Value [®]
Water	Nestle Pure Life: Purified Water	Nestle [®]
Starter culture	FD-DVS YC-380 Freeze dried culture blend at a 1:1 ratio of <i>Lactobacillus delbrueckii subsp. bulgaricus</i> and <i>Streptococcus thermophilus</i>	CHR HANSEN [®]
Pectin	Low methoxyl (LM) pectin.	Gum Technology [®]
Blueberry puree	Natural Blueberry Chunky Variegating Sauce WONF	SENSIENT
<i>L. acidophilus</i>	LYO 100 DCU-S	HOWARU [®]

Table 2. Reconstituted commercial yogurt powder (CYP) and yogurt cultured milk powder (YCMP) formulations

INGREDIENTS	TREATMENTS	
	CYP (g)	YCMP (g)
Commercial Yogurt Powder	948.4	0
Non-fat Dry Milk	0	810
Water	2700	2700
Blueberry Puree	720	720
Citric Acid	0	23.4
Pectin	0	72
Yogurt Starter Culture	0	43

Water was preheated to 40°C then all ingredients were added in to the water. The concentration of freeze-dried yogurt starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is 1% w/w. The mixture was stirred well and transferred to the cooler at 4°C until further analyses without going through the fermentation progress. Yogurt manufacture was replicated 3 times.

For the sensory evaluation, one more treatment of YCMP with *Lactobacillus acidophilus* (YCMPA) was included. In this treatment, 1% w/w of *L. acidophilus* was added into the YCMP and then refrigerated at 4°C. The manufacture process of CYP and YCMP was the same.

2.3 PREPARATION OF MEDIA

2.3.1 Peptone Water

Peptone and water (0.1%) was prepared by dissolving 1g of peptone medium (Bacto™ Peptone, Difco, Dickinson and company, Sparks, MD) in 1L of distilled water, and then autoclaved in 99ml portions at 121°C for 15 minutes.

2.3.2 *Lactobacilli* MRS Agar

The *Lactobacilli* MRS agar for growth of *Lactobacillus delbrueckii ssp. bulgaricus* was prepared according to the instructions given by the manufacturer (Difco™, Dickinson and company, Sparks, MD). 55 g of *Lactobacilli* MRS broth powder and 15 g agar (Fisher Scientific, Fair Lawn, NJ) were added and mixed into 1 L of distilled water. The pH of the mixture was adjusted to 5.2 ± 0.1 using 1N HCL and then autoclaved at 121°C for 15 minutes (Dave and Shah 1996).

2.3.3 *Streptococcus thermophilus* Agar

The ingredients for the *Streptococcus thermophilus* Agar was prepared in the following manner:

10g of tryptone (Becton, Dickinson and Co., Sparks, MD), 10g of sucrose (Amresco, Solon, OH), 5g of yeast extract (Becton, Dickinson and Co., Sparks, MD) and 2g of K₂HPO₄ (Fisher Scientific, Fair Lawn, NJ) were dissolved in 1L of distilled water. The pH of mixture was adjusted to 6.8±0.1 using 1N HCL; after 6mL of 0.5% bromocresol purple (Fisher Scientific, Fair Lawn, NJ) and 12g of agar (Fisher Scientific, Fair Lawn, NJ) were added to the mixture. The medium was then autoclaved at 121°C for 15 minutes (Dave and Shah 1996).

2.4 ANALYTICAL PROCEDURES

2.4.1 Growth of *S. thermophilus* and *L. bulgaricus*

Growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* was measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP, and was determined by pour plate method. 1 g of yogurt samples were diluted to serial appropriated dilution with 99 ml of sterilized 0.1% peptone water (Difco, Deroit, MI). 1 ml of diluted samples were pipetted into petri dishes and then pour plates was applied. Lactobacilli MRS agar was prepared for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* agar was for *Streptococcus thermophilus*. For *Streptococcus thermophilus*, poured plates were incubated aerobically at 37°C for 24 hours and or *Lactobacillus bulgaricus*, poured plates were incubated anaerobically at 43°C for 72 hours (Dave and Shah, 1996, Tharmaraj and Shah, 2003). The colonies were counted after incubation.

2.4.2 E. coli/Coliform and Yeast and Mold Counts

E. coli/Coliform and yeast and mold counts were measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP by using E. coli/Coliform petrifilm (3M™, St. Paul, MN) containing violet red bile agar for E. coli/coliform and yeast and mold

petrifilm (3M™, St. Paul, MN) for yeast and mold. The procedure was performed by weighing 11 g of CYP and YCMP samples and diluting into 99 ml of sterilized 0.1% peptone water (Difco, Detroit, MI) separately. Dilutions of 10^{-1} and 10^{-2} was prepared and 1 ml of the dilutions were plated in duplicate on previously labeled petrifilm and incubated at 32°C for 24 hours for E. coli/coliform and 25°C for 120 hours. The colonies were counted after the incubation.

2.4.3 pH

The pH was measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP by using an Oysters Series pH meter (Extech Instruments, Waltham, MA). The pH meter was calibrated using commercial pH 4.00 and 7.00 buffers (Fisher Scientific, Fair Lawn, NJ) and temperature of the instrument was adjusted to the temperature of sample of $8^{\circ}\text{C} \pm 2$ before reading.

2.4.4 Titratable Acidity (TA)

The titratable acidity was measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP. The titratable acidity was determined by weighing 9 g of yogurt and 5 drops of phenolphthalein as indicator solution were added to the yogurt sample without any blueberry puree because the color of blueberry puree can interfere the identified slight pink color as the end point of titration. The mixtures were titrated by 0.1 N NaOH until the color changed to slight pink and persists for 30 seconds. The volume of NaOH used was recorded.

2.4.5 Apparent Viscosity

The apparent viscosity was measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP by using a viscometer (Brookfield model DV-II and

helipath stand, Brookfield Engineering Lab Inc., Stoughton, MA.USA). Samples were measured at $8^{\circ}\text{C} \pm 2$. A RV1 spindle was used at speed 50 rpm for CMP and a RV4 spindle was used at speed 5 rpm to obtain a torque force. The RV spindle was inserted in the center of the sample at a constant depth of 2 cm from the top level of the sample. The helipath was set in downward motion to cut circular layer at increasing depths of the sample. The size container for the sample was 8 ounce with top diameter 3", bottom diameter 2.3" and height 3.55". The apparent viscosity was determined at $8^{\circ}\text{C} \pm 2$ and was continuous over 33 seconds required to collect 100 data points per replication acquired by the computer using Windgather 32 software (Brookfield Engineering Lab Inc., Stoughton, MA.).

2.4.6 Color

The L^* , a^* , b^* , C^* and h values of color were measured at days of 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after reconstitution of CYP and YCMP by using a colorimeter (Hunter MiniScan® XE Plus portable color spectrophotometer, model No. 45/0-L, serial nr 6666, Hunter Associates Laboratory Inc., Reston, Va., U.S.A) and the Universal software (v4.10). The instrument was standardized using the Hunter lab color reflectance standards (a black standard plate and a white standard plate for serial number 6666). The operating conditions were illuminant D65, 10°observer and 45/0 sensor. An average of 5 values of the L^* , a^* , b^* , C^* and h was taken per replication. According to the applications note (HunterLab, 2008), the following formulas were used:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$h = \arctan \frac{b^{*2}}{a^{*2}}$$

2.4.7 Sensory Evaluation

The sensory evaluation was approved by the LSU Institutional Review Board (IRB) through the exemption number is HE13-12 (Appendix A). The sensory evaluation was conducted with 100 random participants include students and faculty at Louisiana State University. Three treatments, CYP, YCMP and YCMP with 1% w/w added *Lactobacillus acidophilus*(YCMPA) were packed in 2 oz. plastic cups and provided to participants randomly order using identical cups coded with 3-digit random numbers (i.e. 380 for CYP, 519 for YCMP without *Lactobacillus acidophilus* and 778 for YCMP with 1% w/w *Lactobacillus acidophilus*).

Participants were instructed not to talk to others during the evaluation and instructed to clean the palate between each sample with the purified water and non-salted saline crackers. The participants were given a sample of around one oz. per treatment per replication and were asked to evaluate it. The evaluation form consists of a 9-point rating scale, which 1 = dislike extremely and 9 = like extremely, to evaluate appearance, color, aroma, taste, thickness, powderyness, overall like and also questions of acceptability and purchase intent of the products (Appendix B).

2.5 STATISTICAL ANALYSIS

The results for microbial characteristics and physico-chemical characteristics were analyzed using Proc GLM model of Statistical Analysis System (SAS[®] 9.3 program). Differences of Least square means were used to determine significant differences at $P < 0.05$ for main effects (treatment and day) and interaction effect of treatment*day. Significant differences ($P < 0.05$) among the main effects were analyzed using Tukey's adjustment. The results for sensory evaluation was subjected to ANOVA analyzing by SAS[®] 9.3 program, data are presented as mean \pm standard deviation of

the mean. Significant differences between means were determined at $\alpha = 0.05$ using Tukey's adjustment.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 GROWTH

3.1.1 *Streptococcus thermophilus*

The growth of *Streptococcus thermophilus* in reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 1.

1. The treatment*day interaction effect was not significant ($P > 0.05$) while the treatment effect, day effect were significant ($P < 0.05$) (Table 3).

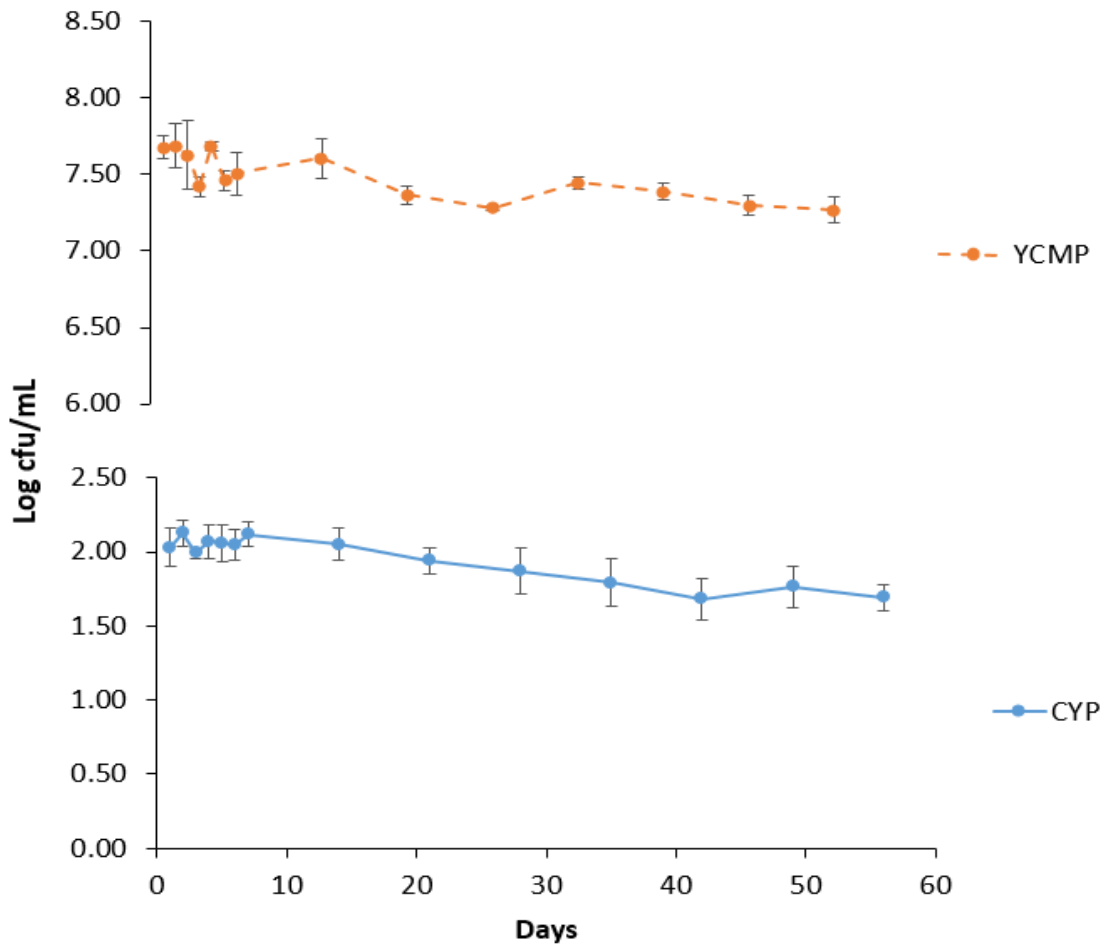


Figure 1. Growth of *Streptococcus thermophilus* in CYP and YCMP during storage period of 8 weeks

Mean of *Streptococcus thermophilus* counts (7.48 log cfu/mL) in YCMP was more than 3 times higher than *Streptococcus thermophilus* counts (1.94 log cfu/mL) in CYP (Figure 1 and Table 4). Considering the commercial yogurt powder was manufactured by spray-drying process, the reason for this significant difference might be low survival rates during spray-drying of the cultures, low stability under storage and the difficulty in rehydrating the product (Peighambaroust, 2011). According to Boza (2004), due to an increase in the lag phase before the onset of growth, spray-dried starter cultures cannot be used for inoculation in dairy fermentations directly.

Table 3. Probability > F (Pr > F) of fixed effects for the growth of *Streptococcus thermophilus* in CYP and YCMP

EFFECT	Pr > F
TREATMENT	< 0.0001
DAY	<0.0001
TREATMENT * DAY	0.0774

Table 4. Least Square Means for the growth of *Streptococcus thermophilus* in yogurt as influenced by CYP and YCMP

Treatment	Viscosity
CYP	1.94 ^B
YCMP	7.48 ^A

^{AB} LSMeans with different letter within the table are significantly different

During the storage period over 8 weeks, the counts of *Streptococcus thermophilus* observed at days 1, 2 and 5 were significantly ($P < 0.05$) higher compared the counts observed at days 28, 35, 42, 49 and 56. The higher mean counts of *Streptococcus thermophilus* were obtained in the initial weeks while the counts decreased in the latter weeks. Michael (2010) also reported that the growth

of *Streptococcus thermophilus* declined from 9 to 8 log cfu/mL in yogurt during storage period of 50 days.

Table 5. Least Square Means for the growth of *Streptococcus thermophilus* in CYP and YCMP as influenced by day

Days	<i>S. thermophilus</i>
1	4.86 ^{AB}
2	4.91 ^A
3	4.81 ^{ABCD}
4	4.75 ^{ABCD}
5	4.87 ^{AB}
6	4.76 ^{ABCD}
7	4.80 ^{ABC}
14	4.83 ^{ABC}
21	4.65 ^{BCDE}
28	4.57 ^{DE}
35	4.62 ^{CDE}
42	4.56 ^{DE}
49	4.49 ^E
56	4.48 ^E

^{ABC} LSMeans with different letter within the table are significantly different

3.1.2 *Lactobacillus delbrueckii ssp. bulgaricus*

The growth of *Lactobacillus delbrueckii ssp. bulgaricus* in reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 2. The treatment*day interaction effect, treatment effect and day effect were significant ($P < 0.05$) (Table 6).

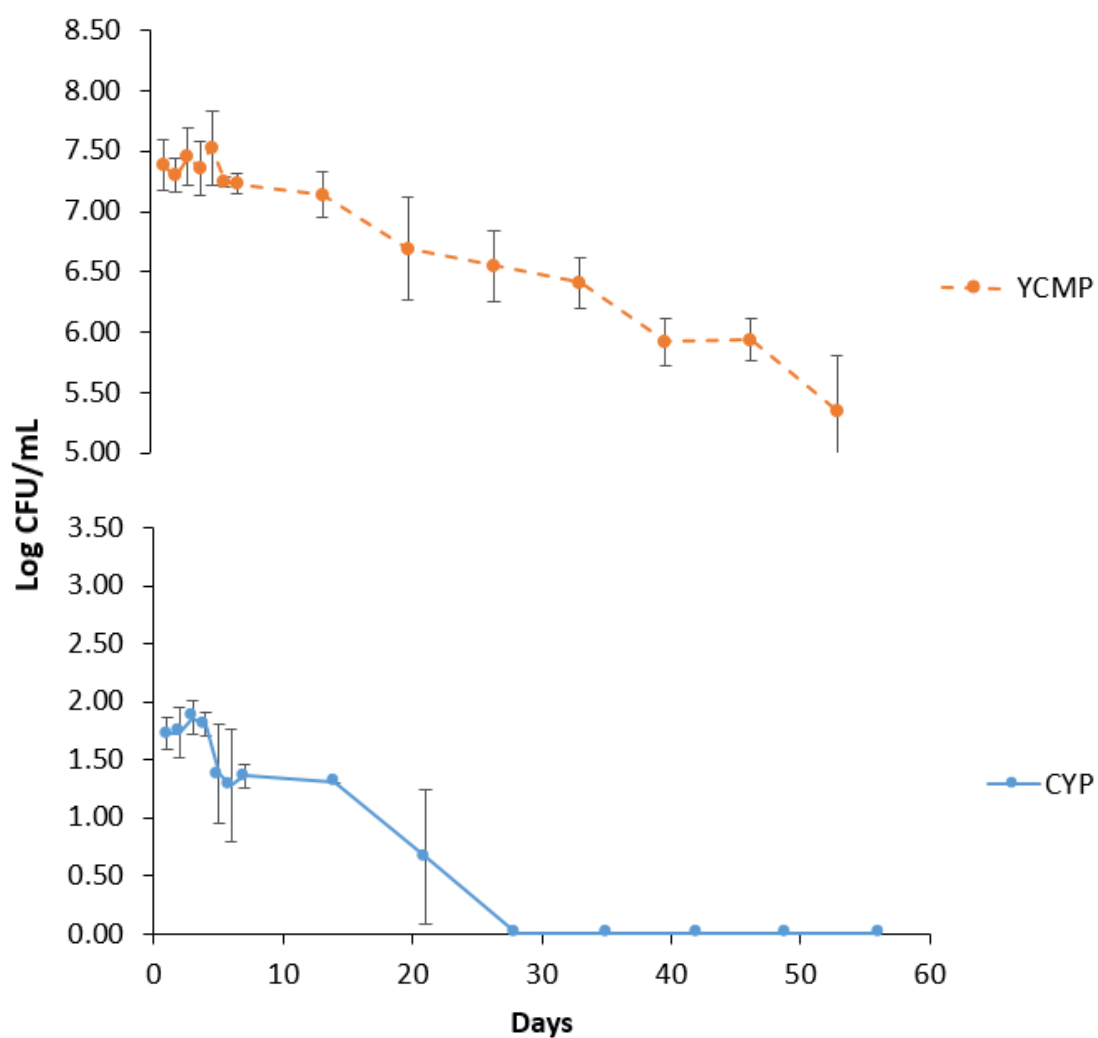


Figure 2. Growth of *Lactobacillus bulgaricus* in CYP and YCMP during storage period of 8 weeks

Table 6. Probability > F ($Pr > F$) of fixed effects for the growth of *Lactobacillus bulgaricus* in CYP and YCMP

EFFECT	Pr > F
TREATMENT	< 0.0001
DAY	<0.0001
TREATMENT * DAY	0.0060

The YCMP *Lactobacillus bulgaricus* counts were significantly ($P < 0.05$) higher than the CYP over 8 weeks storage (Figure 2 and Table 6). In the spray-drying process, numbers of *Lactobacillus bulgaricus* decreased with increased outlet or inlet air temperature, and atomizing air pressure (Kim and Bhowik, 1990). Bielecka and Majkowska (2000) reported that the best survival of *L. bulgaricus* was 13.7-15.8% and *S. thermophilus* was 51.6-54.7% at outlet temperature ranges 70-75°C. Therefore, the original commercial yogurt powder contained a few amount of *L. bulgaricus* and *S. thermophilus*.

Table 7. Least Square Means for the growth of *Lactobacillus bulgaricus* as influenced by CYP and YCMP during the storage of 8 weeks

Days	<i>Lactobacillus bulgaricus</i>	
	CYP	YCMP
1	1.73 ^{CD}	7.38 ^H
2	1.73 ^{CD}	7.30 ^H
3	1.86 ^D	7.45 ^H
4	1.80 ^D	7.36 ^H
5	1.38 ^C	7.52 ^H
6	1.28 ^C	7.25 ^H
7	1.36 ^C	7.23 ^H
14	1.30 ^C	7.14 ^H
21	0.67 ^B	6.69 ^G
28	0.00 ^A	6.55 ^G
35	0.00 ^A	6.41 ^G
42	0.00 ^A	5.92 ^F
49	0.00 ^A	5.94 ^F
56	0.00 ^A	5.35 ^E

^{ABC} LSMeans with different letter within the table are significantly different

During the storage period over 8 weeks, the counts of *Lactobacillus bulgaricus* in CYP observed during the first three weeks (days 1, 2, 3, 4, 5, 6, 7, 14 and 21) were significantly ($P < 0.05$) higher compared to the counts observed during the last five weeks (days 28, 35, 42, 49, 56). The viable counts decreased to 0 cfu/mL from day 28 onwards. The counts of *Lactobacillus bulgaricus* in YCMP observed during the first two weeks (days 1, 2, 3, 4, 5, 6, 7 and 14) were significantly ($P < 0.05$) higher compared to the counts observed during the last six weeks (days 21, 28, 35, 42, 49 and 56). Furthermore the counts observed at days 21, 28 and 35 were significantly ($P < 0.05$) higher than counts observed at days 42, 49 and 56 (Figure 2 and Table 7). The higher counts of *Lactobacillus bulgaricus* in CYP were obtained in the first three weeks in the range of 1.86 log cfu/mL to 0.67 log cfu/mL. The viable counts decreased to 0 cfu/mL from the fourth week onwards (Table 7). Similarly, the higher counts of *Lactobacillus bulgaricus* in YCMP were obtained in the first two weeks in the range of 7.52 log cfu /mL to 7.14 log cfu /mL. The viable counts decreased to the lowest at 56 days (Table 7). Michael (2010) also reported that the growth of *Lactobacillus bulgaricus* declined from 8 to 3 log cfu/mL in yogurt during storage period of 50 days

3.1.3 E. coli/coliform

There were no *E. coli*/coliform bacteria observed both in CYP and YCMP during the storage period of 8 weeks. Also there were no *E. coli*/coliform counts exist in the ingredients of CYP and YCMP. Therefore, there was no health problem related to *E. coli*/coliform in these reconstituted yogurt products.

3.1.4 Yeast and mold

The growth of yeast and mold in reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 3. The

treatment*day interaction effect and treatment effect were not significant ($P > 0.05$) while the day effect was significant ($P < 0.05$) (Table 8).

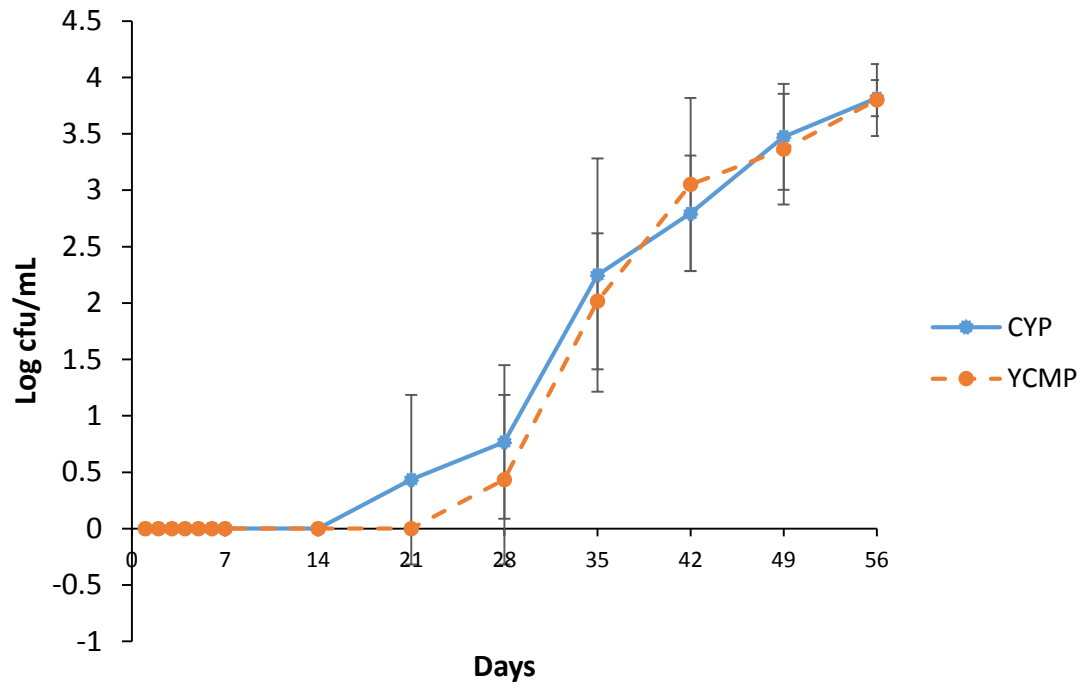


Figure 3. Growth of yeast and mold in CYP and YCMP during storage period of 8 weeks.

Table 8. Probability > F ($Pr > F$) of fixed effects for the growth of yeast and mold in CYP and YCMP

EFFECT	Pr > F
TREATMENT	0.4763
DAY	<0.0001
TREATMENT * DAY	0.9932

Table 9. Least Square Means for the growth of yeast and mold (log cfu/mL) in CYP and YCMP as influenced by day

Days	Yeast and mold
1	0.00 ^D
2	0.00 ^D
3	0.00 ^D
4	0.00 ^D
5	0.00 ^D
6	0.00 ^D
7	0.00 ^D
14	0.00 ^D
21	0.22 ^D
28	0.60 ^D
35	2.13 ^C
42	2.92 ^{BC}
49	3.42 ^{AB}
56	3.81 ^A

^{ABC} LSMeans with different letter within the table are significantly different

Yeast and mold were not presented both in CYP and YCMP in the first two weeks after they reconstituted. Yeast and mold was observed from day 21 (0.22 log cfu/mL), then the counts steadily increased during the storage period and reached to the highest counts at 56 days. The counts of yeast and mold observed at days 35, 42, 49 and 56 were significantly ($P < 0.05$) higher compared to the counts observed at the first four weeks (days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28) (Table 9). Since no pasteurization was involved in the manufacture of CYP and YCMP, a few active cells of yeast and mold might originate from contaminated ingredients and exist in the reconstituted yogurt. It was reported that yeast standards for acceptability of non-fermented dairy products, like cream and butter, and fermented dairy products, such as yogurt, were stated as less than 10 yeast

cells/g (but preferably less than 1 cell/g). Based on the yeast and mold data, reconstituted yogurts had a shelf life of 28 days. Spoilage becomes evident when the yeast population reaches 10^5 - 10^6 cells/g (Fleet, 1990).

CYP spoiled one week earlier than YCMP (Figure 3), but no yeast and mold were obtained in the first two weeks after the reconstitution (Figure 3). Since no one would leave the reconstituted yogurt for more than one week, the interest in this study was in one week after reconstitution, but did extended study to know what would happen if reconstituted products were left that long. Both of CYP and YCMP were safe in the first week of refrigerated storage.

3.2 APPARENT VISCOSITY

The apparent viscosity of reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 4. The treatment*day interaction effect and day effect were not significant ($P > 0.05$) while the treatment effect was significant ($P < 0.05$) (Table 10).

Table 10. Probability > F (Pr > F) of fixed effects for the apparent viscosity of CYP and YCMP

EFFECT	Pr > F
TREATMENT	<0.0001
DAY	0.3097
TREATMENT * DAY	0.2927

Table 11. Least Square Means for the apparent viscosity (cP) of yogurt as influenced by CYP and YCMP

Treatment	Viscosity
CYP	75.26 ^B
YCMP	8714.62 ^A

^{AB} LSMeans with different letter within the table are significantly different

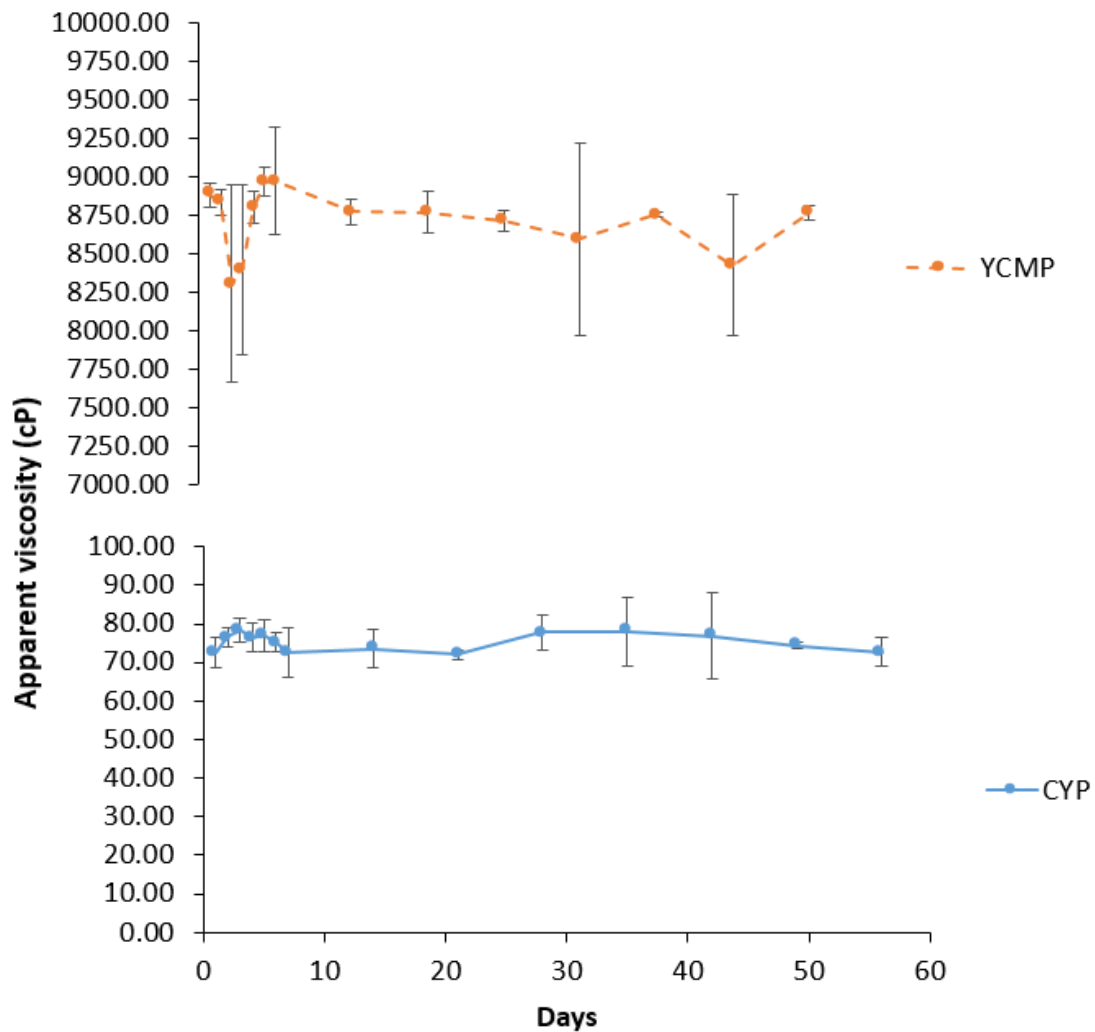


Figure 4. Apparent viscosity of CYP and YCMP during storage period of 8 weeks

Mean of apparent viscosity of YCMP (8714.62 cP) was significantly ($P < 0.05$) higher than apparent viscosity of CYP (75.26 cP) (Table 11). The lacking of pectin of CYP caused its viscosity

under 80 cP which was watery. The texture of CYP was unstable forming 2 distinct layer of settled solids and serum on top. Flavored yogurt drinks are made along with pectin to improve and stabilize viscosity (Tamime and Robinson, 1985). In Basak and Ramaswamy's (1994) study, pectin and strawberry concentrate had a considerable effect of the flow behavior with yield stress of stirred yogurt. As for the effect of day, there were no significantly difference in viscosity of CYP and YCMP respectively during the storage period of 8 weeks.

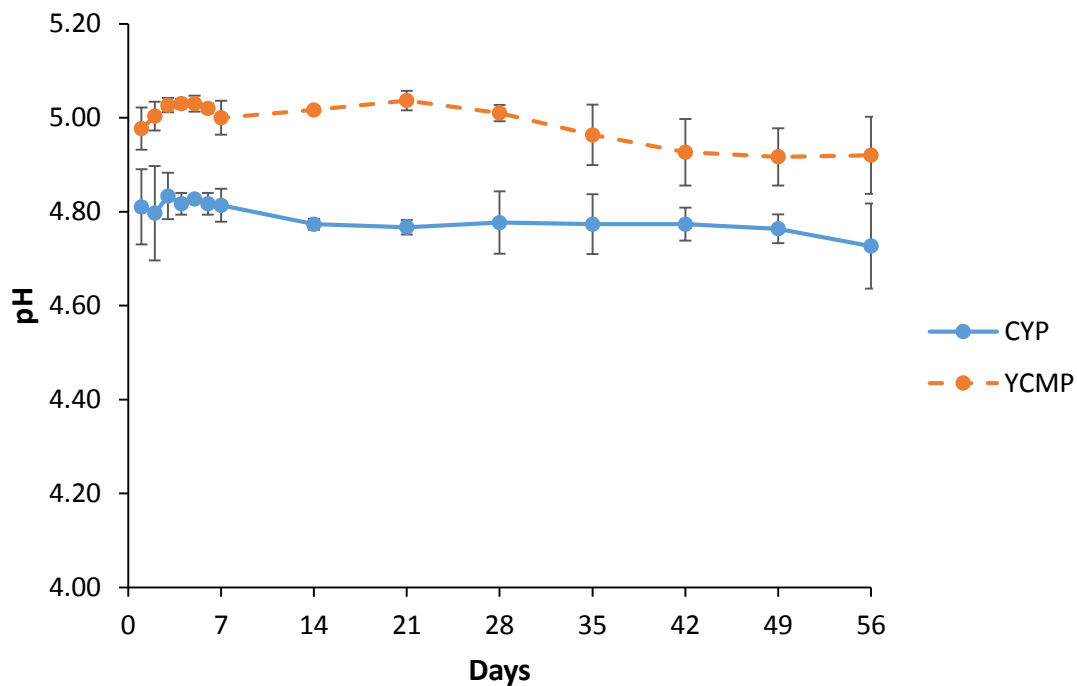


Figure 5. pH of CYP and YCMP during storage period of 8 weeks.

3.3 pH

The pH of reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 5. The treatment*day interaction

effect was not significant ($P > 0.05$) while treatment effect and day effect were significant ($P < 0.05$) (Table 12).

Table 12. Probability $> F$ ($Pr > F$) of fixed effects for pH of CYP and YCMP

EFFECT	Pr > F
TREATMENT	<0.0001
DAY	0.0033
TREATMENT * DAY	0.6421

Table 13. Least Square Means for the pH of yogurt as influenced by CYP and YCMP

Treatment	pH
CYP	4.79 ^A
YCMP	5.00 ^B

^{ABC} LSMeans with different letter within the table are significantly different

Mean of pH value of YCMP (5.00) was significantly ($P < 0.05$) higher than pH value of CYP (4.79) (Table 13). Regarding the day effect, the pH values observed at day 5 was significantly ($P < 0.05$) higher compared to the pH observed at days 42, 49 and 56. Gueimonde *et. al.* (2003) reported a decrease in pH over storage when they studied the quality of plain yogurt stored at 4°C for 44 days. A decrease in pH during yogurt shelf life is expected as a result of the activity of yogurt starter cultures. (Damin *et. al.*, 2009). Since the reconstituted yogurt did not undergo incubation and the fermentation process, their pH (4.8) was higher than regular yogurt (4.6).

Table 14. Least Square Means for the pH of CYP and YCMP as influenced by Day

Days	pH
1	4.89 ^{AB}
2	4.90 ^{AB}
3	4.92 ^{AB}
4	4.92 ^{AB}
5	4.96 ^A
6	4.92 ^{AB}
7	4.91 ^{AB}
14	4.90 ^{AB}
21	4.90 ^{AB}
28	4.89 ^{AB}
35	4.87 ^{AB}
42	4.85 ^B
49	4.84 ^B
56	4.82 ^B

^{ABC} LSMeans with different letter within the table are significantly different

3.4 TITRATABLE ACIDITY

The titratable acidity of reconstituted commercial yogurt powder (CYP) and reconstituted yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 6. The treatment*day interaction effect was not significant ($P > 0.05$) while the treatment effect and day effect were significant ($P < 0.05$) (Table 15).

Table 15. Probability > F (Pr > F) of fixed effects for titratable acidity in CYP and YCMP

EFFECT	Pr > F
TREATMENT	0.0008
DAY	<0.0001
TREATMENT * DAY	0.5605

Table 16. Least Square Means for the titratable acidity of yogurt as influenced by CYP and YCMP

Treatment	TA
CYP	1.27 ^A
YCMP	1.25 ^B

^{AB} LSMeans and with different letter within the table are significantly different

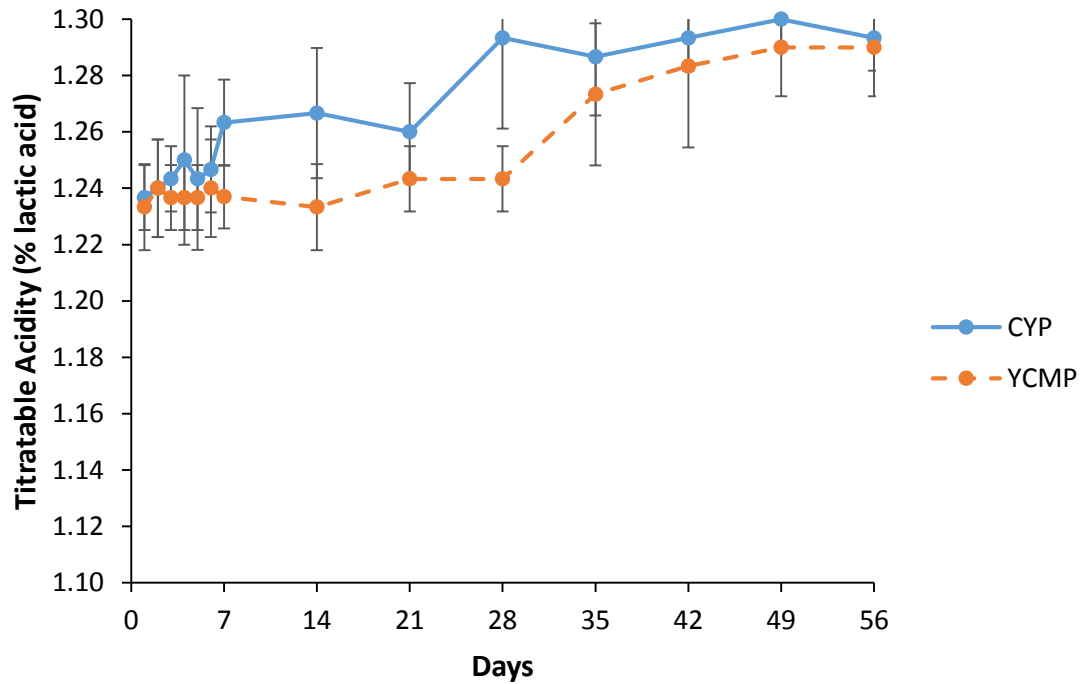


Figure 6. Titratable acidity of CYP and YCMP during storage period of 8 weeks.

Mean of titratable acidity of CYP (1.27%) was significantly ($P < 0.05$) higher compared to YCMP (1.25%) (Table 16). Regarding the day effect, the titratable acidity observed during the first three weeks (days 1, 2, 3, 4, 5, 6, 7, 14 and 21) were significantly ($P < 0.05$) lower than the titratable acidity observed during the last three weeks (days 42, 49 and 56). The titratable acidity increased from 1.23 to 1.29 during 8 weeks refrigerated storage (Table 17). Titratable acidity increased during the storage period might because of the decrease of pH. A decrease in pH during yogurt shelf life is expected as a result of the activity of starter cultures (Damin *et.al.*, 2009).

Table 17. Least Square Means for the Titratable acidity of CYP and YCMP as influenced by Day

Days	TA
1	1.23 ^C
2	1.23 ^C
3	1.23 ^C
4	1.24 ^C
5	1.23 ^C
6	1.24 ^C
7	1.25 ^{BC}
14	1.25 ^{BC}
21	1.25 ^{BC}
28	1.27 ^{ABC}
35	1.28 ^{AB}
42	1.28 ^A
49	1.30 ^A
56	1.29 ^A

^{ABC} LSMeans with different letter within the table are significantly different

3.5 COLOR

3.5.1 L* (Lightness)

The L* value of reconstituted commercial yogurt powder and reconstituted yogurt cultured milk powder during storage of 8 weeks is shown in Figure 7. The treatment*day interaction effect was not significant ($P > 0.05$) while the treatment effect and day effect were significant ($P < 0.05$) (Table 18).

Mean of L* value of YCMP (49.87) was significantly ($P < 0.05$) higher than the L* value of CYP (47.24) (Table 19). Regarding the day effect, L* decreased from 52.85 to 46.84. The L* obtained at day 1 showed to be significantly ($P < 0.05$) higher than L* obtained at days 5, 14, 21, 42, 49 and 56 (Table 20).

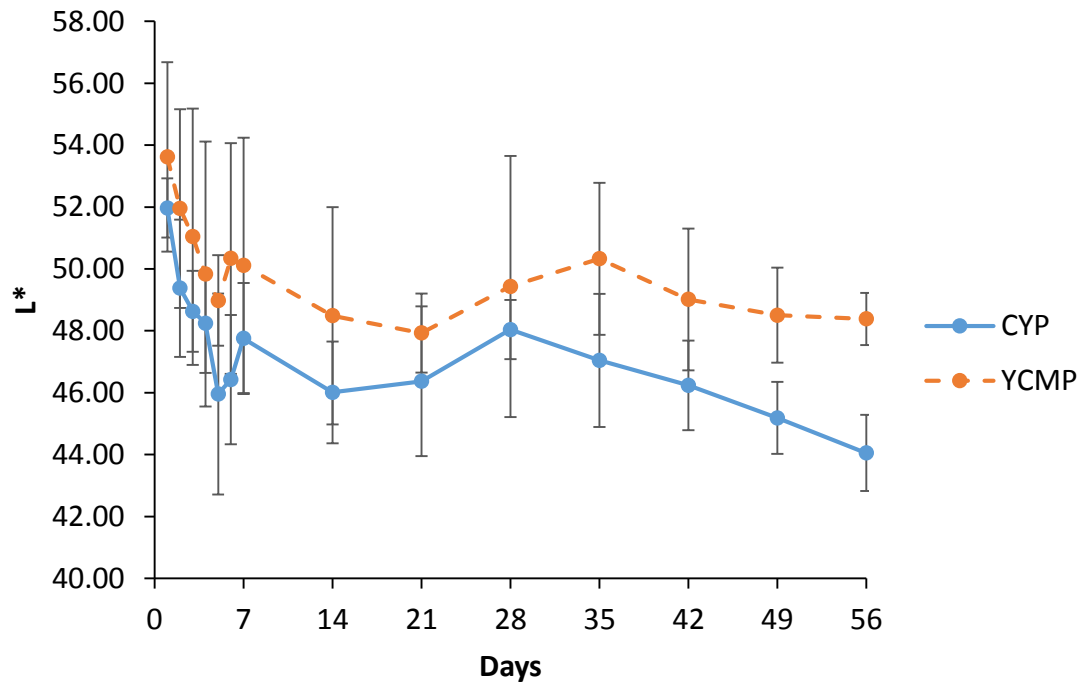


Figure 7. Measurement of L* of CYP and YCMP during storage period of 8 weeks

Table 18. Probability > F (Pr > F) of fixed effects for color of CYP and YCMP

EFFECT	L*	a*	b*	C*	h
TREATMENT	<0.0001	<0.0001	<0.0001	0.0045	<0.0001
DAY	0.0044	0.9859	<0.0001	0.4739	<0.0001
TREATMENT*DAY	0.9991	0.9354	0.0207	0.6425	0.0087

Table 19. Least Square Means for color of CYP and YCMP as influenced by treatment

Treatment	L*	a*	b*	C*	h
CYP	47.24 ^A	4.83 ^A	-2.09 ^A	5.28 ^A	336.83 ^A
YCMP	49.87 ^B	4.07 ^B	-2.62 ^B	4.90 ^B	327.43 ^B

^{ABC} LSMeans with different letter within the table are significantly different

Table 20. Least Square Means for the color of CYP and YCMP as influenced by Day

Day	L*	b*	h
1	52.85 ^A	-2.83 ^C	328.81 ^C
2	50.67 ^{AB}	-2.81 ^C	328.75 ^C
3	49.84 ^{AB}	-2.86 ^C	327.21 ^C
4	49.04 ^{AB}	-2.65 ^{BC}	328.59 ^C
5	47.52 ^B	-2.54 ^{ABC}	330.28 ^{BC}
6	48.38 ^{AB}	-2.66 ^{BC}	328.45 ^C
7	48.94 ^{AB}	-2.62 ^{BC}	328.98 ^C
14	47.25 ^B	-2.51 ^{ABC}	330.50 ^{BC}
21	47.15 ^B	-2.35 ^{ABC}	331.90 ^{ABC}
28	48.74 ^{AB}	-2.33 ^{ABC}	332.32 ^{ABC}
35	48.69 ^{AB}	-1.81 ^{AB}	338.98 ^A
42	47.63 ^B	-1.71 ^{AB}	338.46 ^{AB}
49	46.84 ^B	-1.70 ^{AB}	337.59 ^{AB}
56	46.22 ^B	-1.59 ^A	338.99 ^A

^{ABC} LSMeans with different letter within the table are significantly different

3.5.2 a* (Red-green axis)

The a* of reconstituted commercial yogurt powder and reconstituted yogurt cultured milk powder during storage of 8 weeks is shown in Figure 8. The treatment*day interaction effect and day effect were not significant ($P > 0.05$) while the effect treatment effect was significant ($P < 0.05$) (Table 18).

Mean a* value of both CYP and YCMP were positive indicating that they were in red color space.

The a* of YCMP (4.07) was significantly ($P < 0.05$) lower than the a* of CYP (4.83) (Table 19)

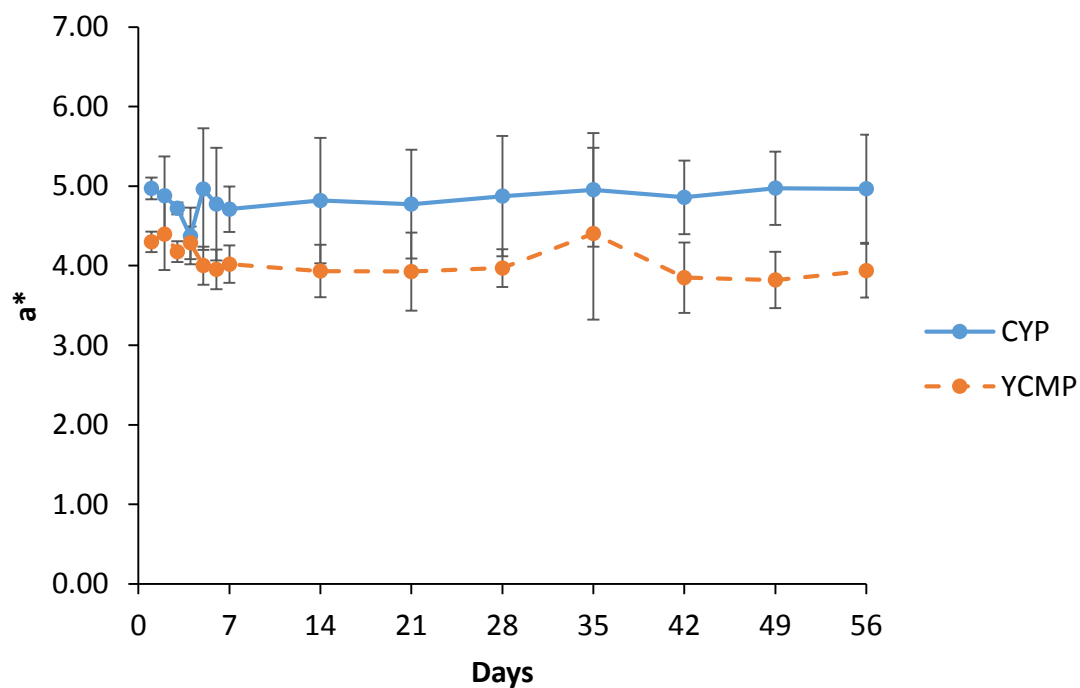


Figure 8. Measurement of a* of CYP and YCMP during storage period of 8 weeks

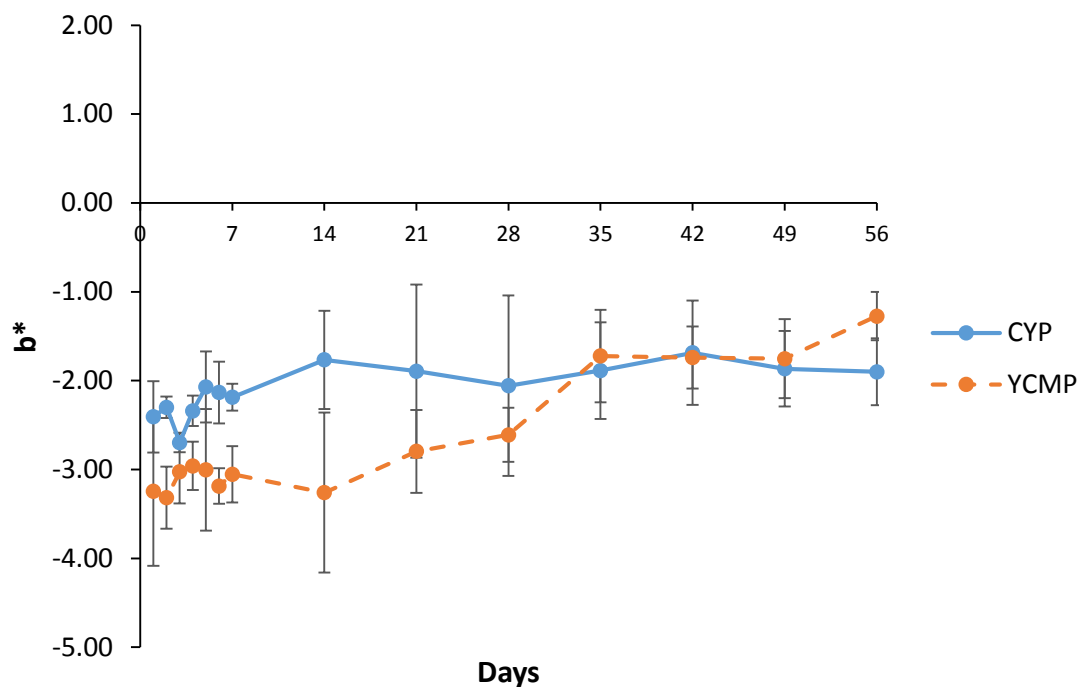


Figure 9. Measurement of b^* of CYP and YCMP during storage period of 8 weeks

3.5.3 b^* (Yellow-blue axis)

The b^* of commercial yogurt powder (CYP) and yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 9. The treatment*day interaction effect, treatment effect and day effect were significant ($P < 0.05$) (Table 18).

The b^* value of both CYP and YCMP were negative indicating that they were in blue color space. At days 1, 2 and 14, the b^* value of YCMP were significantly ($P < 0.05$) lower than the b^* value of CYP (Table 21). During the storage period over 8 weeks, the b^* value of YCMP observed at days 1, 2 and 14 were significantly ($P < 0.05$) lower than the b^* value observed at days 35, 42, 49 and 56 (Table 21).

Table 21. Least Square Means for color of as influenced by CYP and YCMP during the storage of 8 weeks

Days	b*	
	CYP	YCMP
1	-2.41 ^B	-3.25 ^A
2	-2.30 ^B	-3.32 ^A
3	-2.70 ^{AB}	-3.03 ^{AB}
4	-2.34 ^B	-2.96 ^{AB}
5	-2.07 ^{BC}	-3.00 ^{AB}
6	-2.13 ^B	-3.19 ^{AB}
7	-2.19 ^B	-3.05 ^{AB}
14	-1.77 ^{BC}	-3.26 ^A
21	-1.89 ^{BC}	-2.80 ^{AB}
28	-2.06 ^{BC}	-2.61 ^{AB}
35	-1.89 ^{BC}	-1.72 ^{BC}
42	-1.69 ^{BC}	-1.74 ^{BC}
49	-1.87 ^{BC}	-1.75 ^{BC}
56	-1.90 ^{BC}	-1.27 ^C

^{ABC} LSMeans with different letter within the table are significantly different

3.5.4 C* (Chroma/saturation)

Chroma is the aspect of color in the Munsell color system by which a sample appears to differ from a gray of the same lightness or brightness and that corresponds to saturation of the perceive color. The C* value of commercial yogurt powder (CYP) and yogurt cultured milk powder (YCMP) during storage of 8 weeks is shown in Figure 10. The treatment*day interaction effect and day effect were not significant ($P > 0.05$) while the treatment effect was significant ($P < 0.05$) (Table 18). The C* value of YCMP (4.90) was significantly ($P < 0.05$) lower than the C* value of CYP (5.28) (Table 19)

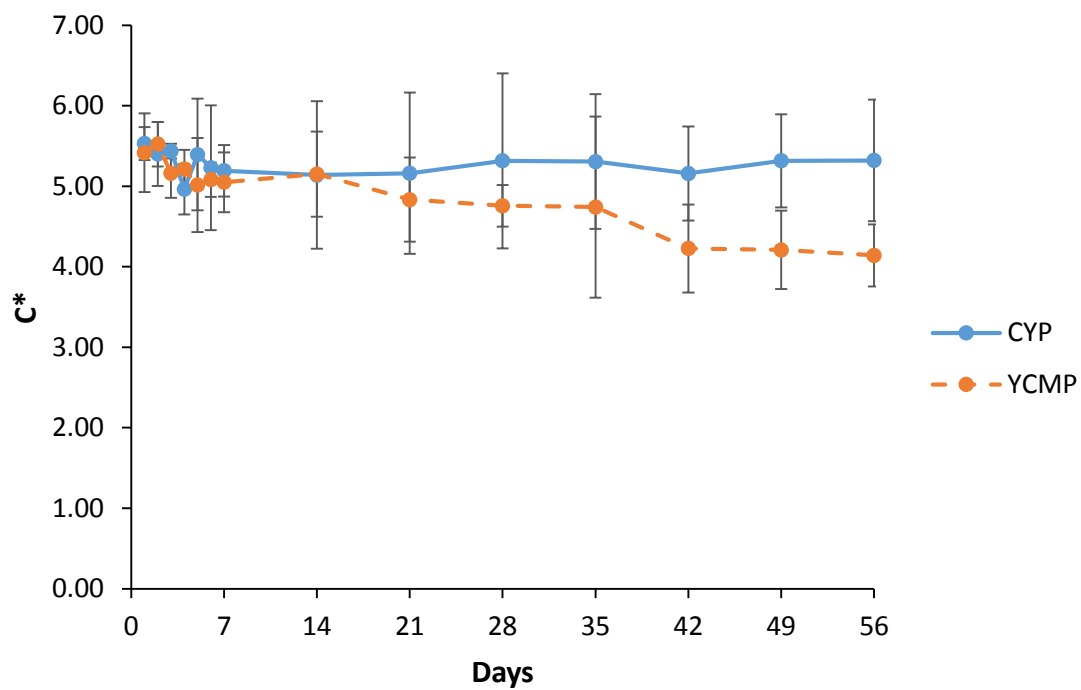


Figure 10. Measurement of C^* of CYP and YCMP during storage period of 8 weeks

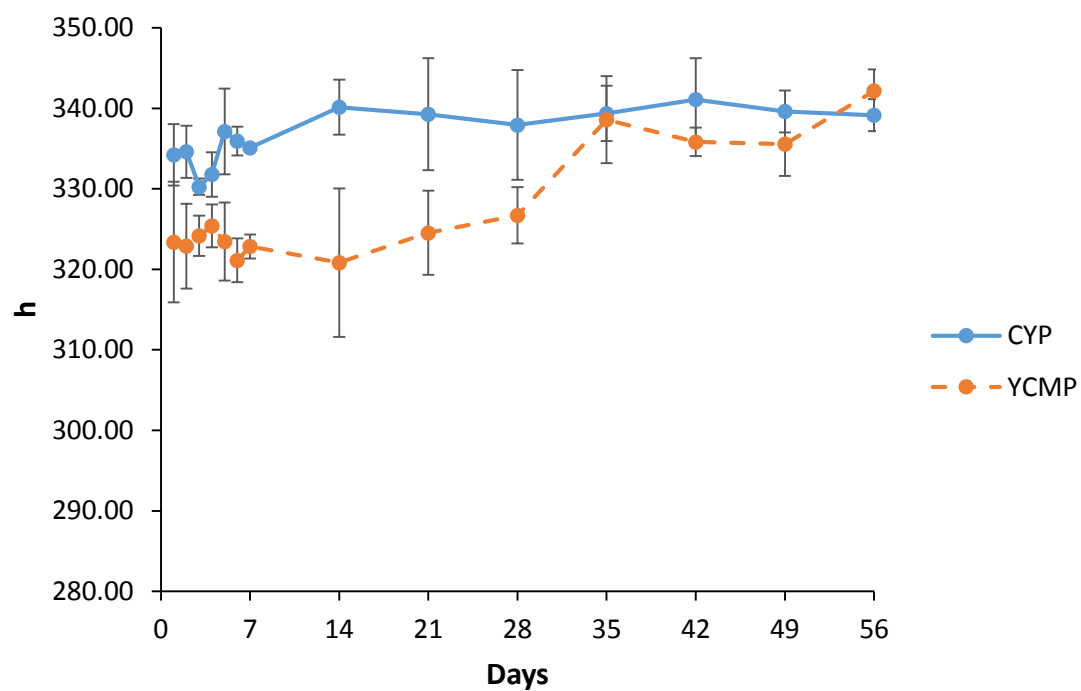


Figure 11. Measurement of h of CYP and YCMP during storage period of 8 weeks

3.5.5 h (hue)

The h value of commercial yogurt powder and yogurt cultured milk powder during storage of 8 weeks is shown in Figure 11. The treatment *day interaction effect, treatment effect and day effect were significant ($P < 0.05$) (Table 18).

At days 2, 6, 7, 14, 21 and 28, the h^* value of YCMP were significantly ($P < 0.05$) lower than the h value of CYP (Table 22). During the storage period 8 weeks, the h values of YCMP observed at the first four weeks (days 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28) were significantly ($P < 0.05$) lower than the h value observed at days 35 and 56 (Figure 11 and Table 22).

Table 22. Least Square Means for color of h for CYP and YCMP

Days	h	
	CYP	YCMP
1	334.23 ^{BC}	323.39 ^{AB}
2	335.10 ^{BC}	322.89 ^A
3	330.26 ^B	324.17 ^{AB}
4	331.79 ^{BC}	325.40 ^{AB}
5	337.12 ^{BC}	323.44 ^{AB}
6	335.93 ^{BC}	321.11 ^A
7	335.10 ^{BC}	322.84 ^A
14	340.10 ^C	320.84 ^A
21	339.26 ^C	324.53 ^{AB}
28	337.94 ^C	326.70 ^{AB}
35	339.37 ^C	338.57 ^C
42	341.10 ^C	335.83 ^{BC}
49	339.61 ^C	335.57 ^{BC}
56	339.15 ^C	342.15 ^C

^{ABC} LSMeans with different letter within the table are significantly different

The purple color of yogurt was obtained from blueberry puree which contains bluish colorant and anthocyanin. Cinbas and Yazici (2008) reported that color values of yogurt with blueberries and sugar added did not change significantly throughout storage of 20 days. According to Jing and Giusti (2005), anthocyanins could interact with many components in milk matrices such as protein and lactic acid. Colors from natural sources have been reported to lose tinctorial strength of fade over storage period (Krammerer et. al., 2006). In Wallace and Giusti's (2008) study, reaction of anthocyanins could be retarded at the low temperature (4°C) so that yogurt color remains stable.

3.6 SENSORY EVALUATION

The sensory evaluation of reconstituted commercial yogurt powder (CYP), yogurt cultured milk powder without *Lactobacillus acidophilus* (YCMP) and yogurt cultured milk powder with *Lactobacillus acidophilus* (YCMPA) was conducted with 100 random people. Means and standard deviation for all sensory attributes (appearance, color, aroma, taste, thickness, powderyness and overall like) of CMP, YCMP and YCMPA are shown in Figure 12. Probabilities for fixed effect of sensory attributes are shown in Table 23. There was a significant ($P < 0.05$) difference among treatments.

Table 23. Probability > F Value (Pr>F) for fixed effect of sensory attributes of CMP, YCMP and YCMPA

EFFECT	Appearance	Color	Aroma	Taste	Thickness	Powderyness	Overall Like
TREATMENT	<0.0001	0.0009	0.0025	<0.0001	<0.0001	<0.0001	<0.0001

In terms of all attributes (appearance, color, aroma, taste, thickness, powderyness and overall like), CYP obtained significantly lowest scores when compared to the other two reconstituted yogurt (Table 24). Compare to yogurt cultured milk powder without *Lactobacillus acidophilus*, yogurt

cultured milk powder with 1% w/w *Lactobacillus acidophilus* had significantly higher scores on appearance, taste, powderyness and overall like. There were no significant difference in color, aroma and thickness between YCMP and YCMPA (Table 24). The overall like scores indicated that YCMP and YCMPA were preferred over CYP (Table 24).

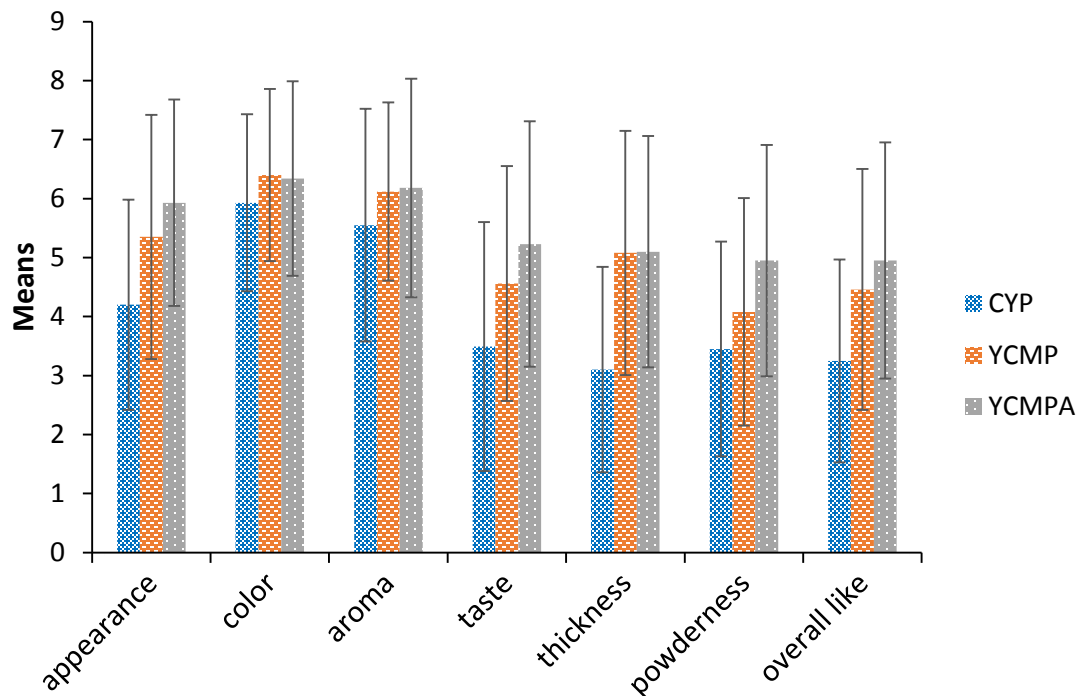


Figure 12. Mean scores for sensory attributes of CYP, YCMP and YCMPA

According to Routray (2011), aroma and taste are the most important sensory characteristics of yogurt. Since no pectin existed in CYP, the texture of CYP was not stable and the scores of thickness, taste and appearance were significantly ($P < 0.05$) lowest amount the other two. According to Olson *et. al.* (2008) Yogurt inoculated with 2.33g/100g *L. acidophilus* had the highest amount of syneresis so that it is to be expected that these yogurts had the lowest appearance/color scores. However, the reconstituted yogurt in this study did not go through the fermentation, taste of YCMP and YCMPA might be influenced by the flavor of non-fat dry milk. Non-fat dry milk may possess a slight cooked or heated flavor. Some common flavor defects of

non-fat dry milk include scorched, stale, storage, old and oxidized (Rankin *et. al.*, 2009). Using citric acid to replace lactic acid as increasing sourness of yogurt might also have influence the taste of YCMP and YCMPA.

Table 24. Means an standard deviation for sensory attributes for CMP, YCMP and YCMPA

TREATMENT	Appearance	Color	Aroma	Taste	Thickness	Powderyness	Overall Like
CYP	4.20 ^A ± 1.78	5.93 ^A ± 1.50	5.55 ^A ± 1.97	3.49 ^A ± 2.11	3.10 ^A ± 1.74	3.45 ^A ± 1.82	3.25 ^A ± 1.72
YCMP	5.35 ^B ± 2.07	6.40 ^B ± 1.46	6.12 ^B ± 1.51	4.56 ^B ± 1.99	5.08 ^B ± 2.07	4.08 ^B ± 1.93	4.46 ^B ± 2.04
YCMPA	5.93 ^C ± 1.75	6.34 ^B ± 1.65	6.18 ^B ± 1.85	5.23 ^C ± 2.08	5.10 ^B ± 1.96	4.59 ^C ± 1.96	4.95 ^C ± 2.00

^{ABC} LSMeans with different letter within the table are significantly different

Reconstituted yogurt acceptability frequency is shown in Figure 13. Acceptability of YCMP (54%) and YCMPA (59%) were more than twice that of the CYP (22%). Reconstituted yogurt purchase intent frequency of CYP, YCMP and YCMPA before knowing they contained probiotics which provide health benefits is shown in Figure 14. The purchase intent of YCMP (24%) and YCMPA (24%) had more than three times that of the CYP (7%). Reconstituted yogurt purchase intent frequency of YCMP and YCMPA after knowing they contained probiotics which provide health benefits is shown in Figure 15. The purchase intent of YCMP increased from 24% to 42% and purchase intent of YCMPA increased from 24% to 46%.

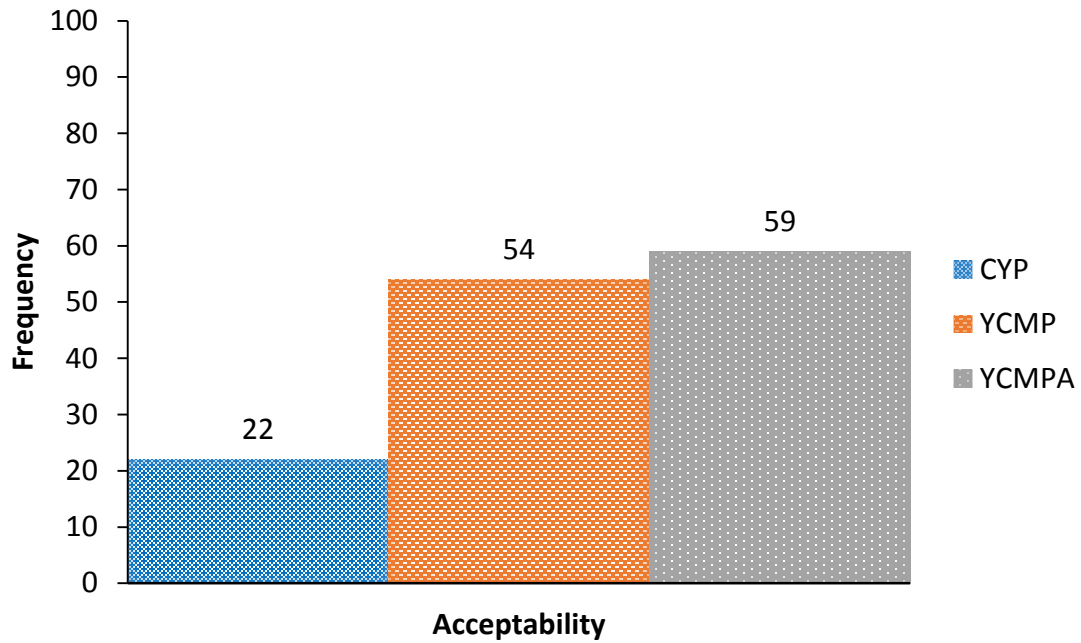


Figure 13. Frequency for acceptability of CYP, YCMP and YCMPA

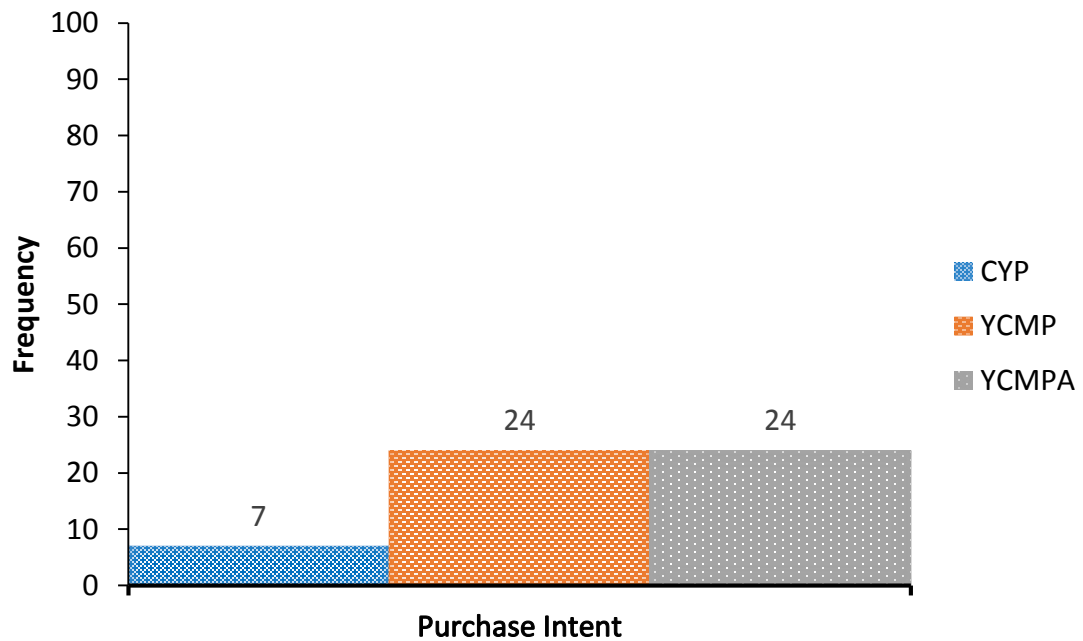


Figure 14. Frequency for purchase intent of CYP, YCMP and YCMPA before knowing they contained probiotics which provide health benefits

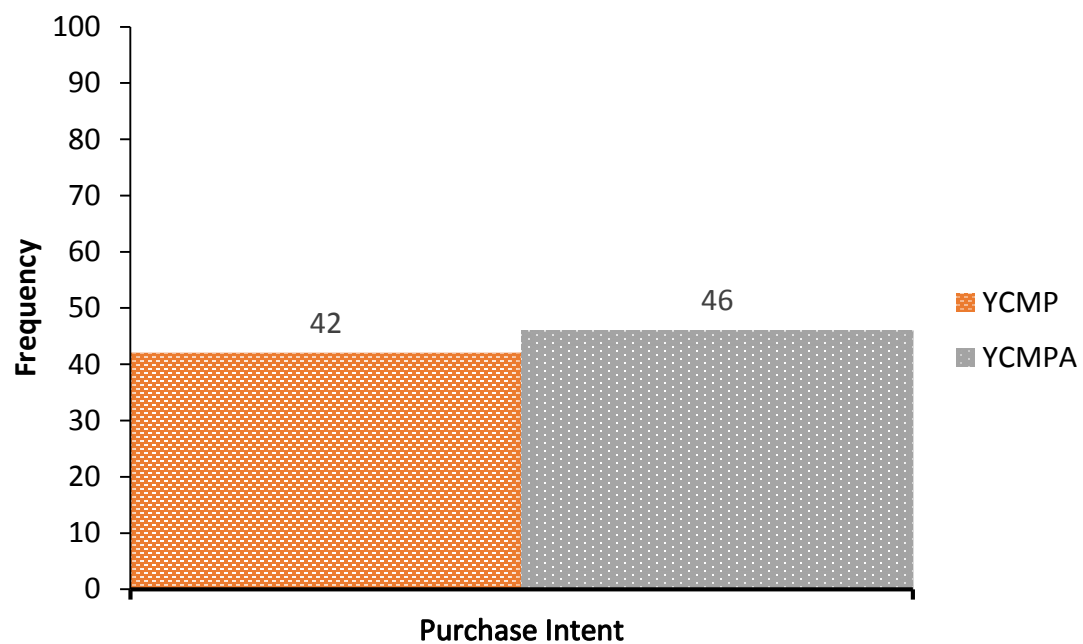


Figure 15. Frequency for purchase intent of YCMP and YCMPA after knowing they contained probiotics which provide health benefits

CHAPTER 4: CONCLUSION

Results of this study showed that YCMP had 5 log cfu/ml higher counts of *Streptococcus thermophilus* compared to the control (CYP) at 56 days. Also, *Lactobacillus bulgaricus* counts of YCMP at 28 days was 6.55 log cfu/ml and at 56 days was 5.35 log cfu/ml while the CYP at 28 days onwards had no counts. CYP cannot meet the recommended yogurt culture bacteria concentration of 6-8 log cfu/g which can be met by YCMP. No coliform were observed at 56 days for both CYP and YCMP. Apparent viscosity of YCMP was significantly higher compared to CYP. YCMP had significantly higher pH but significantly lower TA compared to CYP. YCMP had significantly higher L* and lower a*, b* C* and h compared to CYP. YCMP had significantly higher appearance, sensory color, aroma, taste and thickness scores compared to CYP. Consumer acceptability of YCP (54%) was more than twice that of CYP (22%). Yogurt cultured milk powder had a markedly better culture bacterial counts, physico-chemical and sensory characteristics compared to commercial available yogurt powder.

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APPENDIX A. CONSENT FORM FOR CONSUMER EVALUATION

RESEARCH CONSENT FORM

APPROVED BY
LSU AG CENTER
IRB AS #E13-12
ON 9-13-2013

I, _____, agree to participate in the research entitled "Yogurt cultured milk powder as a substitute for yogurt powder" which is being conducted by the School of Animal Sciences at Louisiana State University, phone number (225)-578-4411.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated in my school. I can withdraw my consent at any time without penalty of loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. A total of at least 100 people will participate in this research. For this particular research, about a 10 minute participation will be required.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on the acceptance of yogurt cultured milk powder. The benefits that I may expect from it are a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: Coded samples of yogurts will be placed in front of me and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risks: The only risk that can be envisioned is an allergic reaction to milk and lactose intolerance. However, because it is known to me beforehand what type of food to be tested, the situation can normally be avoided.
5. The results of this participation will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Michael Keenan, Chairman, Institutional Review Board, (225) 578 1708, mkeenam@agcenter.lsu.edu

Keyanush J. Anyane

Signature of Investigator

Signature of Participant

Date: _____

Witness: _____

APPENDIX B. QUESTIONNAIRE FOR CONSUMER EVALUATION

Sample # _____

Date _____

PLEASE EVALUATE THE PRODUCT AND CHECK THE SPACE THAT BEST REFLECTS YOUR FEELING ABOUT THE PRODUCT. BETWEEN SAMPLES, YOU ARE REQUIRED TO RINSE YOUR PALATE WITH WATER AND CHEW ON UNSALTED CRACKER WHICH CAN BE EXPECTORATED.

1. How would you rate the overall **APPEARANCE** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

3. How would you rate the **AROMA** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

4. How would you rate the **TASTE** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

5. How would you rate the **TEXTURE (THICKNESS)** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

6. How would you rate the **TEXTURE (POWDERYNESS)** of this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

7. OVERALL, how much do "**LIKE**" this product?

Dislike	Dislike	Dislike	Dislike	Neither like	Like	Like	Like	Like
Extremely	Very Much	Moderately	Slightly	nor dislike	Slightly	Moderately	Very Much	Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

8. Is this product **ACCEPTABLE**? Yes [] No []

9. Would you **BUY** this product if it were commercially available? Yes [] No []

10. Would you **BUY** this product if you know it contained probiotics which provide health benefits? Yes [] No []

VITA

Lijie Song was born in Kunming, Yunnan, People's Republic of China in June, 1989. She finished her undergraduate studies at Jimei University, Xiamen, People's Republic of China in June, 2011. She received her Bachelor's Degree in Food Science and Engineering. In August 2011, she was admitted by Department of Food Science of Louisiana State University and Agricultural and Mechanical College to pursue graduate study. In August 2012, she was transferred to School of Animal Science of Louisiana State University and Agricultural and Mechanical College. Currently, she is a candidate for Master's degree in Animal, Dairy and Poultry Science with concentration in dairy science, which will be awarded in December 2013.