UTILIZATION OF WHEY POWDER IN THE ENCAPSULATION OF *LACTOBACILLUS ACIDOPHILUS* BY SPRAY DRYING FOR THE PRODUCTION OF PROBIOTIC YOGURT

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ABSTRACT

UTILIZATION OF WHEY POWDER IN THE ENCAPSULATION OF *LACTOBACILLUS ACIDOPHILUS* BY SPRAY DRYING FOR THE PRODUCTION OF PROBIOTIC YOGURT

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Yogurt is a valuable functional food and has an important market worldwide. Yogurt is made by fermentation of milk with lactic cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Since yogurt contains viable bacterial cultures, its shelf life related to the viability of the cultures and lactic acid formation, is a critical problem for the food industry. In order to prevent undesirable effects of artificial additives, natural additives are preferred to prolong shelf life of yogurt. Natural additives such as milk powder, whey powder, lactose, inulin, casein, starch and others may be added to yogurt. The main objective of this study was to determine the effect of whey powder and probiotic encapsulated whey powder on the shelf life of yogurt, the viability of the yogurt bacteria and the probiotic bacteria.

In the first part of the study, optimization of spray drying conditions for the encapsulation of probiotic *Lactobacillus acidophilus* in whey powder was done by Response Surface Methodology. Optimized conditions were found as 140°C for inlet temperature, 10 rpm for pump rate (with liquid flow rate of 0.485 L/h), and 0.83:0.17 for whey powder to arabic gum ratio. 48.36% production efficiency and 93.95% encapsulation efficiency were achieved in spray drying to produce probiotic encapsulated powder with optimized conditions. These whey powders were analyzed for the viability of probiotic cultures and their particle size distributions. The yield for viability of L. acidophilus in encapsulation was found as 95%. While the yield for viability of free *L. acidophilus* was found as 75.46%, the yield for viability of encapsulated *L. acidophilus* after being exposed to simulated gastrointestinal was found as 89.16%. The second part of the study consisted of yogurt analysis. Yogurt samples prepared with or without encapsulated probiotics and the shelf life was analyzed over 28 days of storage at 4°C. *L. acidophilus* can survive during 28 days of storage (as cell number 10^8 CFU/g) in yogurt containing encapsulated probiotic whey powder on the contrary to free cells cannot survive. At the end of 28 days of storage and even 50 days of storage, there was not seen mold and yeast formation in yogurt with whey powder. Sensory analysis showed that the addition of whey powder did not affect the yogurt properties negatively.

Keywords: yogurt, probiotic, shelf life, whey, encapsulation, spray drying, response surface methodology, *L. acidophilus*.

ÖZ

PEYNİR ALTI SUYU TOZUNDA *LACTOBACILLUS ACIDOPHILUS*'UN PÜSKÜRTMELİ KURUTMA İLE ENKAPSÜLASYONU VE PROBİYOTİK YOĞURT ÜRETİLMESİ AMACI İLE KULLANIMI

Değirmenci, Cansu

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Yoğurt dünya çapında önemli bir pazara sahip olan değerli bir fonksiyonel besindir. Yoğurt sütün mayalanması sonucu elde edilir. Yoğurdun mayalanması için süt *Streptococcus thermophilus* ve *Lactobacillus delbrueckii bulgaricus* içeren canlı laktik kültürleri ile aşılanır. Yoğurt yaşayan bakteri kültürleri içerdiğinden dolayı, kültürlerin canlılığı ve laktik asit oluşumuyla ilgili raf ömrü, gıda sanayisi için önemli bir sorundur. Yapay katkı maddelerinin istenmeyen etkilerini önlemek amacıyla, doğal katkı maddeleri yoğurdun raf ömrünü arttırmak için tercih edilmektedir. Yoğurda eklenen doğal katkı maddelerine örnek olarak süt tozu, peynir altı suyu tozu, laktoz, inulin, kazein ve nişasta gösterilebilir.

Bu çalışmanın temel amacı, peynir altı suyu tozunun yoğurt raf ömrü ve yoğurt bakterileri ile probiyotik bakterilerin canlılığı üzerindeki etkisini belirlemektir. Çalışmanın ilk kısmında, probiyotik bakteri *L. acidophilus*'un peynir altı suyu tozunda kapsülasyonu için gereken püskürtmeli kurutucu koşullarının Cevap Yüzeyi Yöntemi ile optimizasyonu yapılmıştır. Püskürtmeli kurutmada optimum koşullar giriş sıcaklığı için 140°C, pompa hızı için 10 rpm (485 mL/s sıvı akış hızı ile) ve peynir altı suyunun arabik gama oranı için 0.83:0.17 bulunmuştur. Probiyotik kapsüllü peynir altı suyu tozu üretimi için optimum koşullarda püskürtmeli kurutucuda üretim yapıldığında ise % 48.36 üretim verimi ve % 93.95 kapsülasyon verimine ulaşılmıştır. Bu peynir altı suyu tozlarında, probiyotik bakterinin canlılığı ve parçacık boyutu dağılımı analizleri yapılmıştır. Kapsülasyonda *L. acidophilus*'un canlılık verimi %95 olarak bulunmuştur. Simüle edilmiş mide-bağırsak sisteminde serbest olan probiyotiğin canlılık verimi % 75.46 olarak bulunmuş iken, kapsüllenmiş probiyotiğin canlılık veriminde %89.16'ya ulaşılmıştır. Çalışmanın ikinci kısmı yoğurt analizlerini içermektedir. Yoğurt örnekleri kapsüle edilmiş ve edilmemiş probiyotik ve farklı eklemelerle hazırlanmıştır ve 28 gün boyunca 4°C'de depolanarak raf ömrü analizleri yapılmıştır. *L. acidophilus,* serbest probiyotik içeren yoğurtların aksine, kapsüle edilmiş probiyotik içeren peynir altı suyu tozunun eklendiği yoğurtlarda 28 gün depolama boyunca canlılığını korumuştur (10^8 KOB/g). 28 gün depolama sonunda ve hatta 50 gün depolama sonucunda bile peynir altı suyu tozu içeren yoğurtlarda maya ve küf oluşumu gözlenmemiştir. Bunlara ek olarak yapılan duyusal analizlerde, peynir altı suyu tozu eklemenin yoğurdun duyusal özelliklerine olumsuz etki göstermediği bulunmuştur.

Anahtar kelimeler: yoğurt, probiyotik, raf ömrü, peynir altı suyu, enkapsülasyon, püskürtmeli kurutma, cevap yüzeyi yöntemi, *L. acidophilus*.

To my beloved family,

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LIST OF ABBREVIATIONS

AG: arabic gum

E: efficiency

GI: gastrointestinal tract

LA: *Lactobacillus acidophilus*

LB: *Lactobacillus bulgaricus*

P-WP/AG: probiotic encapsulated whey powder and arabic gum

ST: *Streptococcus thermophilus*

TS: Turkish Standards

v/v: volume per volume

w/v: weight per volume

WP: whey powder

WP/AG: Whey powder and arabic gum mixture

CHAPTER 1

INTRODUCTION

1.1. Milk and Milk Products

Milk is defined as a fluid that is secreted by the mammalian glands of females for the nourishment of their infants. Being the primary source of nutrition, it is a most valuable and natural food material.

Milk can be obtained from cow, sheep, goat, buffalo, horse, yak or camel. The basic nutritional components of milk are energy, water, protein, fat, carbohydrate, vitamins, minerals and minor biological proteins and enzymes. However, milk content may be varied according to the source. The compositions of milk from different sources is given in Table 1.1.

Milk consumption has an important role for having healthy life due to its association with nutrition. It provides significant amount of essential vitamins and minerals for human diet. According to a research which was done in UK, consuming a glass of milk (186 ml) every day increases the intake of daily requirements for human diet Table 1.2 shows contribution of milk to nutrient intake (%) for different age range results handled in the research in UK.

Table 1.1 Composition of milk from different sources per 100 g of milk (Mc Cane et al., 2007)

	Unit	Cow	Sheep	Goat	Buffalo
Water	g	87.8	83	88.9	81.1
Protein	g	3.2	5.4	3.1	4.5
Fat	g	3.9	6	3.5	8
--Saturated Fatty acids	g	2.4	3.8	2.3	4.2
-- Monounsaturated fatty acids	g	1.1	1.5	0.8	1.7
-- Polyunsaturated fatty acids	g	0.1	0.3	0.1	0.2
Lactose	g	4.8	5.1	4.4	4.9
Cholesterol	mg	14	11	10	8
Calcium	mg	120	170	100	195
Energy	kcal	66	95	60	110
	kJ	275	396	253	463

Table 1.2 Contribution of milk to nutrient intake (%) (Gregory et al., 1995)

	Age 1.5-4.5	Age 4-18	Age 19-64	Age 65+
Protein	24	11	9.7	13.6
Vitamin A	30.5	10.9	7	13
Riboflavin (B2)	37.5	24.8	23.3	26.7
Vitamin B6	22.5	9.4	8.3	14
Vitamin B12	39.7	36.6	29.1	24.7
Calcium	46.9	28.3	26.2	36.1
Iodine	39.6	33.7	28.5	25.5
Magnesium	22.2	10.3	8.1	13.5
Phosphorus	32.7	16.4	15.1	21.2
Potassium	25.6	13	10.8	15.7
Zinc	25	13	9.7	13.6

Milk product is defined as a “product obtained by any processing of milk, which may contain food additives, and other ingredients functionally necessary for the processing” in the Codex Alimentarius.

The aim of processing milk is to obtain products with longer shelf life. Milk products varies according to the traditions of people in different parts of the world. In Turkey, yogurt, ayran, cheese, milk powder, kefir, butter and cream are mostly preferred milk products. In order to make shelf life longer milk products, fermentation technique is used. This method also gives particular flavor to product. The first examples of products made by using fermentation method are cheese, bread and wine in Neolithic ages. Yogurt and other fermented milk products were followed by vinegar, alcoholic beverages and pickles (Shurtleff & Aoyagi, 2007).

Fermentation is a technique that used in food chemical processing to convert carbohydrate to alcohol or organic acids by the help of microorganisms (yeast or bacteria) under anaerobic conditions. By different microorganism, different products can be obtained. In the Figure 1.1 fermented milk products are classified.

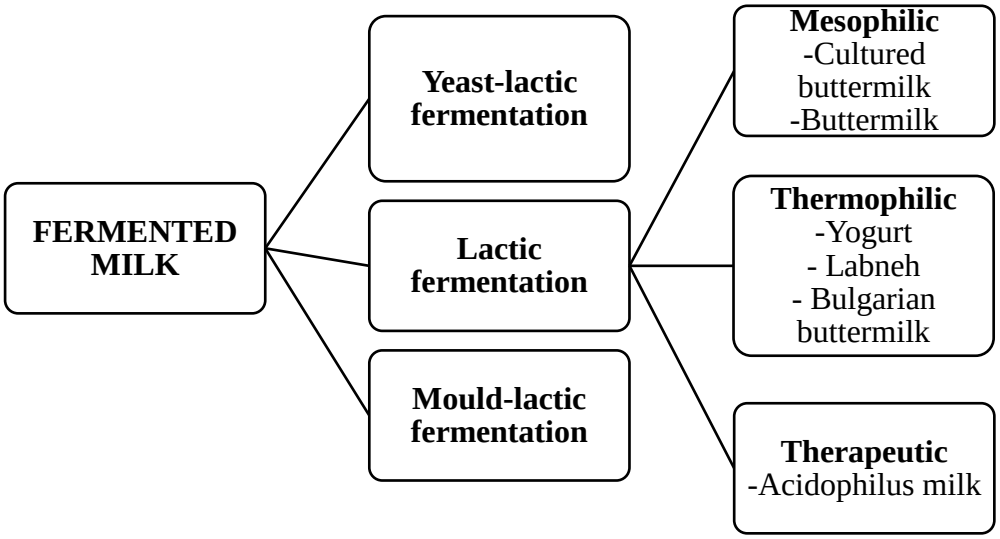

Figure 1.1 Fermented milk classification (Tamime & Robinson, 2007)

1.2. Yogurt

Yogurt is a functional food resulting from the fermentation of homogenized and pasteurized milk in the presence of lactic cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*.

1.2.1 Yogurt Production

Yogurt can be produced as set or stirred yogurt in the industry. In Figure 1.2 the flow chart of yogurt manufacturing can be seen.

Commercial yogurts are manufactured in two ways as set and stirred yogurt. In order to produce set yogurt, the milk is fermented in retail boxes. This method provides a continuous gelled structure in the final product. On the other hand, stirred yogurts are made where the fermentation of milk is done in large incubation tanks. For giving more fluid product, yogurt is stirred to disrupt gelled structure (Haque et al., 2001). Stirred style has disadvantage in reaching desired thickness and consistency of yogurt because only some strains of microorganisms can achieve. Moreover, aroma compounds that occurred at low incubation temperatures are less in compare to high incubation temperatures (Walstra et al., 2006). For that reason, set yogurt production has some advantages in terms of time and temperature of incubation.

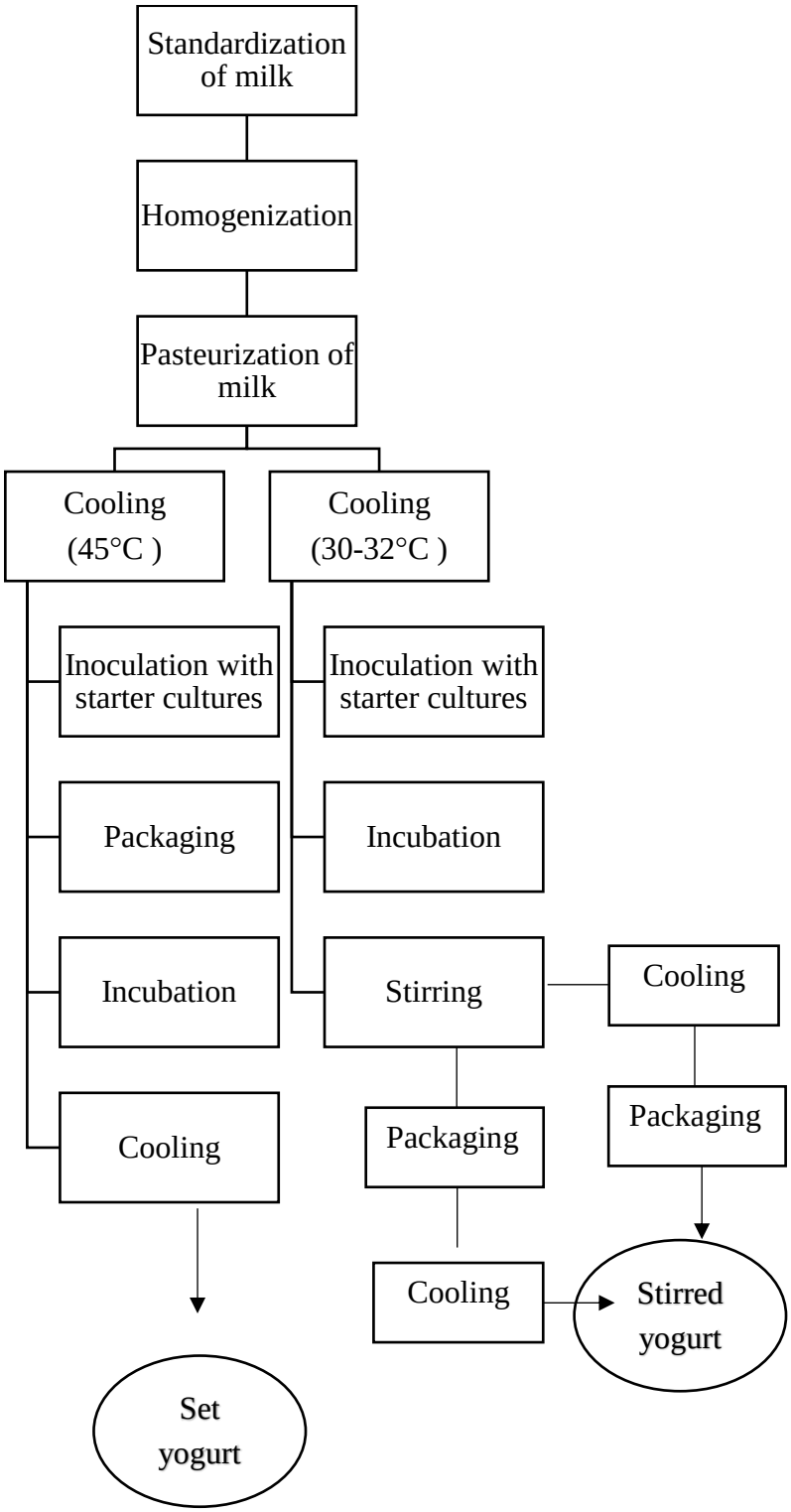

Figure 1.2 Yogurt manufacturing flow chart for set and stirred yogurt (Walstra et al., 2006)

Yogurt manufacturing is detailed in Turkish Standard TS 10935 (April, 1993). The steps of manufacturing yogurt are listed as chart in Figure 1.2. After milk is accepted to factory, it is firstly standardized to adjust fat content for standard and good quality of yogurt production. It is done by separation of excess milk fat or addition of cream to get desired concentration (Tamime & Robinson, 2007). Separation of excess milk fat is done by centrifugal force with the help of density difference between cream and milk. According to Turkish Standard TS 1330 (April, 2006), minimum fat content should be 3.8 % for normal yogurt, 1,5 % for semi-fat yogurt and for non-fat yogurt should be lower than 0,15 %. In standardization step, non-fat solid standardization is also done. Different methods are used in industry to standardize non-fat solid content of milk. These can be listed as evaporation of water in milk, addition of skim milk powder to milk, membrane filtration or addition of condensed milk. Non-fat solid content should be 12% at least for all yogurt types according to TS 1330.

After milk is skimmed and standardized, it is homogenized to inhibit separation of milk fat from milk and obtain uniform product at final stage. The homogenization process is held for breaking up milk fat into smaller portions and consistently dispersed particles. Then, pasteurization is done to eliminate pathogenic microorganisms in milk. Generally, it is done through hot plates in industry. There are different relations of time and temperature and changing according to material used, process and final product requirements. According to TS 10935, high pasteurization method is used and it is held at 90-95°C for 2-3 min or 80-85°C for 20-30 min for yogurt production.

In inoculation or seeding step, milk is cooled down to 42-45°C after pasteurization. Addition of mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* with the ratio 1:1 is done. Inoculation rate is changing from 0.5% to 4% (v/v) (Ribeiro et al., 2014).

After inoculation, temperature of milk is hold at 42-45°C which is the optimum growth temperature for yogurt culture. There are two types of fermentation for yogurt according to Tamime & Robinson (2007).

First fermentation is overnight incubation held at 30°C for 16-18 hours. Second one is held at higher temperature for shorter time, for example at 42°C for 4-5 hours. For production of set yogurt, incubation is done within the package. After filling into yogurt packages, pallets of packages are placed into incubation chamber. For production of stirred yogurt, incubation is done in bulk before mixing and packaging. Fermentation time is required for yogurt pH reaches to 4.8 ± 0.05 (Ribeiro et al., 2014).

Cooling of yogurts are made with system called multi-stage cooling system in industry. The steps of multi-stage cooling system are:

1. Shock cooling: cooling down from incubation temperature to 30°C,
2. Dysgentical stage: yogurts are cooled to 20°C,
3. Lact-less phase: cooling of yogurt to 14.5°C,
4. Holding phase: keeping of yogurts at 2-4°C (White, 1995).

After production, yogurts should be kept at 2-4°C during shelf life.

1.2.2 Component of Yogurt and Effects on Health

The raw material of a food material decide nutritional value of final product. Yogurt becomes very nutritional food thanks to valuable composition of milk. Composition of milk from different sources were given in Table 1.1.1. After fermentation, some of components change and give more beneficial effects to yogurt (Walstra et al., 2006). In Table 1.3, nutrition facts of yogurt from whole milk, skim milk and non-fat milk are given retrieved from USDA Food Composition database.

Table 1.3 Nutrition Facts of Yogurt from whole milk, skim milk and non-fat milk (value per 100g)

	Unit	Yogurt- whole	Yogurt- low fat	Yogurt- non fat
Proximate				
Water	g	81.30	83.56	85.10
Energy	kcal	97	73	59
Protein	g	9	9.95	10.19
Total fat	g	5	1.92	0.39
Carbohydrate	g	3.98	3.94	3.60
Fiber	g	0	0	0
Sugar	g	4	3.95	3.24
Minerals				
Calcium	mg	100	115	110
Iron	mg	0	0.04	0.07
Magnesium	mg	11	11	11
Phosphorus	mg	135	137	135
Potassium	mg	141	141	141
Sodium	mg	35	34	36
Zinc	mg	0.52	0.60	0.052
Copper	mg	0.017	-	-
Manganese	mg	0.009	-	-
Selenium	µg	9.7	-	-
Vitamins				
Vitamin C	mg	0	0.8	0
Thiamin	mg	0.023	0.044	0.023
Riboflavin	mg	0.278	0.233	0.278
Niacin	mg	0.208	0.197	0.208
Pantothenic acid	mg	0.331	-	-
Vitamin B-6	mg	0.063	0.055	0.063
Folate, DFE	µg	5	12	7
Vitamin B-12	µg	0.75	0.52	0.75

Table 1.3 Nutrition Facts of Yogurt from whole milk, skim milk and non-fat milk (value per 100g) (Continued).

	Unit	**Yogurt- whole**	**Yogurt- low fat**	**Yogurt- non fat**
Proximate				
Vitamin A,RAE	µg	2	90	1
Retinol	µg	1	-	-
Carotene, beta	µg	7	-	-
Vitamin A,IU	IU	15	309	4
Vitamin E	mg	0.01	0.04	0.01
Vitamin D (D2 +D3)	µg	0	0	0
Vitamin D	IU	0	0	0
Vitamin K	µg	0	0.2	0
Lipids				
Fatty acid, total saturated	g	2.395	1.230	0.117
Fatty acid, total monounsaturated	g	2.136	0.486	0.053
Fatty acid, total polyunsaturated	g	0.469	0.076	0.012
Fatty acid, total trans	g	0	0.060	0.006
Cholesterol	mg	13	10	5
Other				
Caffeine	mg	0	0	0

1.2.3 Turkish Yogurt Standardization

Standardization is the specification of the uniformity of the goods and services produced to ensure consistent production according to the techniques for the aim of fulfilling the needs of people. The service obtained by producing the same standardized sample is also called standard.

Standards are divided into two: national and international. The Turkish Standards Institute is the supervisory institution that determines which product is produced in accordance with the standard in our country. The producers receive a certificate of conformity from the Turkish Standards Institute for their products. They indicate the TSE mark on their products.

For yogurt, there are several standards. As mentioned before Turkish Standard TS 10935 (April, 1993) is to standardize yogurt manufacturing. The standardized yogurt product should have certain properties and they are given in TS 1330 (April, 2006). Yogurt is divided into five types according to fat ratio: full fat, fatty, semi-fat, low-fat and fat-free. According to TS 1330 sensorial, chemical and microbiological properties are given in table 1.4, 1.5 and 1.6 respectively.

Yogurt should be qualified to receive a total of 16 points, with at least 4 points from each characteristic, with respect to appearance, consistency, smell and taste in accordance with the evaluation criteria given in the Table 1.4 and with at least 4 points from each characteristic. When the yogurt package is opened, a yellowish green liquid covering the upper part and cracks or bubbles on the surface should not be observed.

Table 1.4 Sensory evaluation scores of yogurt (TS 1330, April 2006).

	Score
Appearance	
– Clean, bright, milk-colored, without serum separation, cracks and gas bubbles, homogenous,	5
– Clean, milk-colored, no serum separation, no cracks or gas bubbles,	4 3
– Clean, matt, greyish, few cracks and a small amount of serum separated,	1-2
– Different colors from different colors of milk, many cracks, gas bubbles, containing any foreign substance that is visible,	

Table 1.4 Sensory evaluation scores of yogurt (TS 1330, April 2006) (Continued).

	Score
Consistency	
– The spoon-shaped section is in a thick consistency, uniform, homogenous, after mixing thick fluency, serum is not immediately separated, not easily diffused between palate and tongue,	5
– Received section is in a thick consistency, uniform, homogenous, after mixing thick fluency, serum is rarely separated, minimum diffused between palate and tongue,	4
– Received section is less fluidic, slightly lumpy, smooth after mixing and serum is separated immediately, dispersed when receiving the mouth, slightly lumpy,	3
– Received section is very smooth, inhomogeneous and lumpy, very smooth after mixing, immediately and in excess amount of serum, separated from the tongue and palate, non-retentive, flowable, nonhomogeneous	1-2
Smell	
– Unique sweet smell	4-5
– Non-intrinsic or foreign odor-containing	3
– Unique, alcoholic, burning or foreign smell containing	1-2
Taste	
– Unique light sweet taste,	5
– Slightly sour or slightly sweet	4
– Sour, slightly bitter, slightly moldy, lightly soap or lightly burnt flavored	3
– Extremely sour, bitter, frizzy, soapy burned taste and foreign-flavored	1-2

Chemical properties of yogurt should be suitable with values given in Table 1.5 according to TS 1330 (April 2006).

Table 1.5 Chemical properties of yogurt (TS 1330, April 2006).

	Values			
Properties	**Full fat**	**Semi-fat**	**Low-fat**	**Fat-free**
Fat, % (w/w)	Min 3.8	Min 1.5	Max 1.5	Max 0.15
Total solid non-fat, % (w/w), min	12	12	12	12
Protein, % (w/w), min	4	4	4	4
Titratable acidity (Lactic acid), % (w/w)	Min 0.6 Max 1.6	Min 0.6 Max 1.6	Min 0.6 Max 1.6	Min 0.6 Max 1.6
Peroxidase	Negative	Negative	Negative	Negative
Copper (Cu), mg/kg, max	1	1	1	1
Tin (Sn) , mg/kg, max	200	200	200	200
Lead (Pb) , mg/kg, max	0.02	0.02	0.02	0.02
Mercury (Hg) , mg/kg, max	0.03	0.03	0.03	0.03

Microbiological properties of yogurt should be suitable with values given in Table 1.6 according to TS 1330 (April 2006).

Table 1.6 Microbiological properties of yogurt (TS 1330, April 2006).

Properties	**N**	**c**	**m**	**M**
Coliform bacteria	5	2	9	95
E.coli	5	0	<3	-
Yeast (cfu/g)	5	2	10^1	10^2
Mold (cfu/g)	5	2	10^1	10^2
N= Number of test samples to be analyzed c= The highest number of test specimens (M) can be found m= The upper limit that can be found in the test number (n - c) M= The maximum limit to be found in the number (c) of test samples				

1.3. Probiotics

Probiotics are living organisms, and when they are taken in adequate quantities they can provide microbial balance and improve health of the host. (Fuller, 1989; Awaisheh, 2012). Recently, the definition of the probiotics includes all of the preparations that enhance the health of the organism and can be added to food, food additives or feeds (Uymaz, 2010). In researches, it has been found that probiotics play a therapeutic role by strengthening the immune system, lowering cholesterol, improving lactose tolerance and preventing some cancers (Kailasapathy and Chin, 2000; Sanders et al., 2007). In recent years, as people have given more importance to their health, they want to consume healthier food and the demand for food that resist to diseases has increased. This situation leads to increase interest in probiotics and probiotic food (Kailasapathy, 2009). As noted above, world-wide sales of probiotics rose from $ 21.6 billion in 2010 to $ 24.23 billion within a year (Pedretti, 2013). By 2018, world-wide probiotic sales are projected to rise to $ 44.9 billion (Pedretti, 2013). The proliferation of probiotics in the fields of health, economics, food and increasing market share has led to the increase of scientific researches.

In order for a microorganism to be considered a probiotic, it must have certain properties. These mandatory criteria have been set by the LABIP (Lactic Acid Bacterial Industrial Platform). (Guarner and Schaafsma, 1998, Ewaschuk and Dieleman, 2006). In general, probiotic microorganisms;

- are of human origin,
- do not contain pathogenic properties,
- show resistance to gastric acid and bile salt,
- adhere to intestinal epithelium tissues,
- are alive throughout the gastrointestinal system even for short periods of time,
- are able to produce antimicrobial compounds,
- are able to stimulate the immune response,
- have metabolic ability (cholesterol assimilation, lactase activity, vitamin production)
- are able to resist technological processes. (Uymaz,2010)

Probiotics have a number of positive effects on human health, and new ones are added every day (Kiani, 2006; Lyte, 2011; Bermudez-Brito, et al., 2012). The positive effects on human mental health are quite new. The intensive research on this subject continues (Foster and Neufeld, 2013; Patterson, 2014; Naseribafrouei, et al., 2014; Dinan, et al., 2015; Luna and Foster, 2015; O'Mahony, et al., 2015). According to researches, a summation of health benefits of probiotics and their mechanisms are listed in Table 1.7.

In order to be beneficial, probiotics should have at least concentration of 10^6 cfu/g or cfu/ml within foods. Moreover, 10^8-10^9 cfu/g or cfu/ml of probiotics should be consumed daily to get therapeutic effects.

Table 1.7 Health benefits of probiotics and their mechanism(s).

Health benefit	Mechanism(s)
Prevention of hearth diseases and influence on blood cholesterol level	Absorption of cholesterol by bacteria, deconjugation of bile acid by bacterial acid hydrolases, binding of cholesterol to cell wall of bacteria, diminution of hepatic cholesterol merge, redistribution of cholesterol from plasma to liver by influencing of production of short fatty acids by bacteria
Controlling of irritable bowel syndrome	Transition of gut microbiota and decreasing of intestinal production of gases
Prevention of cancer	Suppression of transformation of carcinogens into active forms, binding and deactivation of mutagenic complex, inhibition growing of pro-carcinogenic bacteria, decreasing the assimilation of carcinogens, enhancement immune system, modify concentration of bile salt
Controlling and prevention of atopic diseases	Controlling of response of immune system
Controlling of incendiary bowel disease	Controlling of immune responses and modulation of gut microbiota
Preventing of urogenital tract illnesses	Producing of antimicrobial material, struggling for adhesion site, competition of pathogens

Table 1.7 Health benefits of probiotics and their mechanism(s) (Continued)

Health benefit	Mechanism(s)
Preventing and treating of *Helicobacter pylori* infection	Producing of antimicrobial materials, stimulating of mucus secreting, competition for adhesion site, stimulating of immune response
Preventing of diarrhea origin by bacteria or virus	Modulating of gut microbiota, producing of antimicrobial materials, competing for adhesion site, stimulating of mucus secreting, modulating of response of immune system
Alleviation of lactose indigestion	β-galactosidase activation on lactose
Reducing of colonic transition time	Influencing on peristalsis through bacteria metabolite production

1.3.1 Probiotic Strains

It is proved that probiotics are used for health benefits. In spite of the fact that different probiotic strains give different benefits. According to Klaenhammer (2001), survival in food, characteristics of fermentation and other performance of probiotics may have varied within different species of probiotics, even in different strains of a specie of probiotic.

1.3.1.1 Selection of strains

Some criteria are needed for a microorganism to be considered as a probiotic. Criteria for selection of probiotics strains are listed in Table 1.8.

Table 1.8 Criteria for selection of probiotic strains

Criteria for selection	Property
Safety	Classification Source Pathogenic properties
Producing and manufacture	Bulk production Storage Constancy and viability Quality
Functionality	Endurance and expansion Acid and bile resistance Adhesion and colony forming properties
Performance	Benefits to health Antimicrobial material production Bioactive substance production

Firstly, a strain have to be specified. The taxonomic classification is done by 16S rRNA sequencing and phylogenetic analysis. The source of species should be normal habitant of targeted species and separated from healthy individual. The strains should have any pathogenic property and have safety requirements. Second criteria for selection of probiotics is about producing and manufacturing. Strains shall be suitable for bulk production and storage. Viability of strains should be longer at high concentration. Their constancy during preparation of culture, storing and delivery should be longer. Quality of strain is also important criteria in terms of processes and adding into food. Third criteria is about strains' capability of endurance and expansion at the site targeted. They must be resistant to acid in order to be survived in gastric way. In addition to acid resistance, strains should be resistant to bile to colonize in the intestinal tract.

The strain should adhere to epithelial cells of human or mucus for colonizing in vivo. In order to utilize therapeutic effect, they should struggle with normal microflora. The final criteria is about strain performance. The strain should have at least one proven health benefit. They should produce antimicrobial material to compete with pathogenic bacteria and bioactive substances like peptides, enzymes or vaccines.

1.4. Lactic Acid Bacteria

Lactic acid bacteria are classified as gram positive, non-spore forming, non-respiring, catalase negative, acid tolerant, rods or cocci microorganisms (Salminen et al., 2004). They produce lactic acid as the major product at end of the fermentation from carbohydrates. Therefore, they are names as lactic acid bacteria. Besides using in fermentation, lactic acid bacteria can be found in gastrointestinal and genitourinary tract of human and animal. They have an important role on health in terms of immunomodulation, resistance to pathogens and intestinal integrity (Vaughan et al., 2005).

Lactic acid bacteria can be classified according to type of fermentation and the product of lactic acid, morphology, optimum temperature of growth, acid and alkaline environment and salt concentration tolerance (Salminen et al., 1998). In table 1.9, the families and genera of lactic acid bacteria are listed.

Table 1.9 Families and genera of LAB

Family	Genus
Aerococcaceae	*Aerococcus*
Carnobacteriaceae	*Carnobacterium*
Enterococcaceae	*Enterococcus,Tetrageonococcus, Vagococcus*
Lactobacillaceae	*Lactobacillus, Pediococcus*
Leuconostocaecae	*Leuconostoc, Oenococcus, Weissella*
Streptococcaceae	*Lactococcus, Streptococcus*

Lactobacillus and *Streptococcus* genera are the most known and used lactic acid bacteria in food industry.

1.4.1 Lactobacillus genus

Lactobacilli belong to gram positive, catalase- negative, facultative anaerobic or microaerophilic, rod shaped and non- spore forming bacteria. Lactobacilli genus is the major part of the lactic acid bacteria. Lactobacilli genus is including a high number of Generally Recognized As Safe (GRAS) species. They can be found in chairs of pairs with varying size and length from (0.5-1.2 x 1-10 μm). Homofermentative and heterofermentative species can be found in Lactobacilli genus (Salvetti et al., 2012).

They are part normal flora of mouth, human and other warm- blooded animal vagina, intestinal tract. Lactobacilli genus is also found in dairy, fish, meat and fermented products. They are playing an important role in fermentation of food and prevention of spoiling food. They can be used as probiotics and starter culture.

Lactobacilli genus has a wide variety of organisms, it is containing over 180 species. Their species can be grouped under 3 different categories. The group 1 includes obligately homofermentative bacteria which are *L. acidophilus, L.delbrueckii, L. salivarius* and *L.helveticus*. The group 2 includes facultatively heterofermentative bacteria which are *L.casei, L. plantarum, L. curvatus* and *L.sakei*. The group 3 includes obligately heterofermentative bacteria which are *L.brevis, L.fermentum, L. reuteri* and *L. buchneri*.

1.4.1.1 *Lactobacillus acidophilus*

Lactobacillus acidophilus is a species belonging to Lactobacilli genus. It is a gram-positive, homofermentative and rod-shaped microorganism. It can ferment sugars into lactic acid as all lactic acid bacteria. It can grow at low pH levels (below 5.0) and has optimum temperature of growth around 37°C. Since being microaerophilic, it can grow aerobically but better grows under anaerobic condition which is containing 5% CO_2, 10% H_2O and 85% N (Robinson, 2005). Some strains of *Lactobacillus acidophilus* is considered to have probiotic properties.

Probiotic strains of *Lactobacillus acidophilus* are used commercially in dairy industries.

Lactobacillus acidophilus has health benefits in human digestive system. The benefits of *L. acidophilus* are:

- Improving blood pressure and cholesterol,
- Fighting with viral, bacterial and fungal infections,
- Improving infant conditions,
- Supplying nutritional benefits,
- Reducing allergic activity,
- Helping digestive system.

With these properties, *Lactobacillus acidophilus* is the most widely studied probiotic. It is also most widely commercially used probiotic in the food industry.

1.5. Yogurt starter cultures

Lactic acid bacteria (LAB) are used as dairy starter cultures by adding to milk in order for production of the fermented product. Fermented milk, yogurt, butter, kefir, cheese etc. can be produced by LAB. Classification is done according to optimum temperature of growth as mesophilic and thermophilic cultures. Optimum temperature of growth is about 30°C for mesophilic cultures. Thermophilic cultures have optimum temperature of growth about 42°C.

In industry, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* are the most widely used thermophilic cultures (Mozzi et al., 2010). The purpose of using two cultures together is the symbiotic relationship between *S. thermophilus* and *L. bulgaricus*. Symbiotic relations is defined as the association between microorganisms in which one microorganism can produce favorable substance for the other. Figure 1.3 shows the symbiotic relationship between them. It is known as *S. thermophilus* goes faster through lag-phase to reduce the redox potential and activate acidity pH from 6.7 to 5.7.

Therefore *S. thermophilus* can support *L. bulgaricus* growth mainly by the production of lactic and formic acid (Tamime and Robinson, 2007). *S. thermophilus* can assimilate oxygen and thus produce carbon dioxide in milk faster. This mechanism creates favorable media for the growth of *L. bulgaricus*.

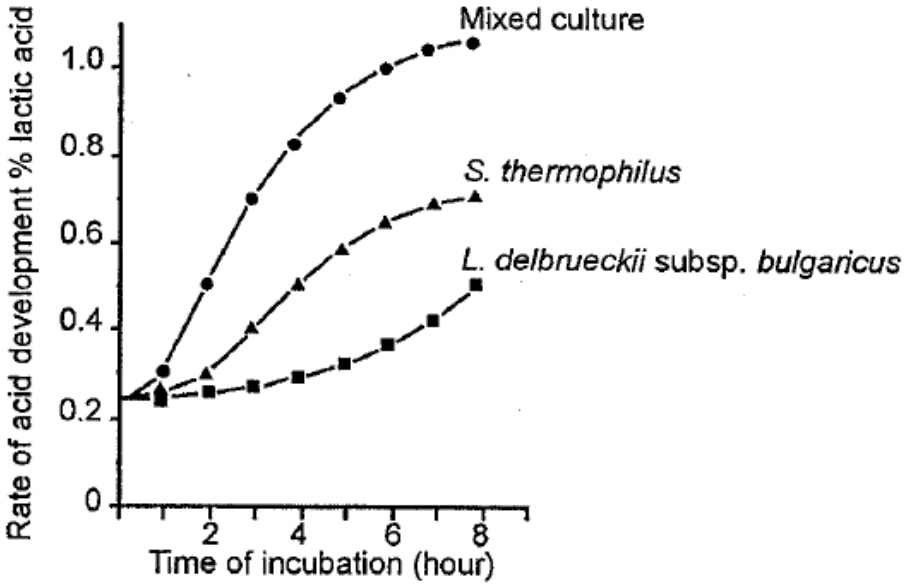

Figure 1.3 Mixed and single cultures propagation in milk at 40°C with 2% (v/v) inoculation (Tamime & Robinson, 2007)

As seen in the figure, these two cultures demonstrate different growth curve during process of fermentation. In the beginning of fermentation *S. thermophilus* is showing a fast growth by the help of *L. bulgaricus* however the number of *S. thermophilus* decreases since it has the ability of low acid production. When the acidity of milk improve, *S. thermophilus* dies faster. Conversely, at high level of acidity *L. bulgaricus* number increases.

Eventually, pH of milk decreases from about 6.4 to 4.6 by the activation of these bacteria. This pH decrease is leading to precipitation of protein molecules at isoelectronic point which is called protein coagulation. The product after this coagulation is named as yogurt. Moreover, this activation between cultures gives yogurt specific taste, texture, smell and aroma.

1.5.1 *Streptococcus thermophilus*

S. thermophilus is used as dairy starter bacteria in yogurt and cheese making. It is the only species from streptococcal that can be used in food industry. It is identified as facultative anaerobic, gram positive, aerotolerant, cocci shaped, non-motile and catalase- negative bacterium. *S. thermophilus* is able to grow at 45°C. It does not generate endospores and *S. thermophilus* does not have oxidase, catalase or cytochrome enzymes. Formerly, *S. thermophilus* was taken into account as a subspecies of *Streptococcus salivarius* because of homologous values of DNA. At present they are considered as two different species according to their heat resistance and using ability of number of carbohydrates. *S. thermophilus* can be used alone or in association with lactobacilli and lactococci. However, for yogurt production *S. thermophilus* is used with *Lactobacillus delbrueckii* ssp. *bulgaricus*.

1.5.2 *Lactobacillus delbrueckii ssp. bulgaricus*

Lactobacillus delbrueckii ssp. *bulgaricus* is gram positive, facultative anaerobic, rod-shaped, non-motile and non-sporulating bacteria. It can be classified as acidophilic bacteria since low pH is required for growing of this bacteria. Similar to *S. thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* is considered as a thermophilic starter culture with optimum growth temperature 42°C or 37°C. During fermentation, it can use lactose to produce aldehydes which gives the aroma to yogurt.

1.6. Microencapsulation

Probiotics must live in the food product and live in the human body after consumption in order for they function, (Kailasapathy, 2009). There are some limiting factors in the use of probiotics in the food industry. Probiotics are prone to lose their vitality during both storage and food processing. Failure to provide physical, chemical, or enzymatic stability conditions causes probiotics to lose their viability (Dias et al., 2015).

In addition to that, probiotics can affect food products negatively in taste, smell and appearance, even if they do not lose their vitality after being added to the food product.

Establishing a physical barrier to enhance endurance within the body during and after shelf life can prevent probiotics from being affected by environmental conditions and can meet sensory consumer expectations. Encapsulation technique is a suitable method for providing that.

Microencapsulation is defined as the process of providing controlled release of packaged material by packing solid, liquid and gaseous substances in small capsules (Champagne and Fustier, 2007; Desai and Park, 2005). The material or mixture in the capsule is called the core, the inner phase or the filler, while the material in the outer part is called the shell, coating, wall material, carrier or membrane (Gharsallaoui et al., 2007). Encapsulation has become attractive in the food industry in recent years because of the qualities such as controlled release of encapsulated substance, limitation of reactivity, protection against environmental, physical, chemical and mechanical stimulants.

1.6.1 Carrier Material

In encapsulating a food component, the most important step affecting the final product is the selection of a suitable carrier material. The choice of carrier material is made according to the core material and properties desired in the final product.

The carrier material;

- can be used in food,
- should be biodegradable,
- should have capability to create a barrier between the core material and the external environment,
- should have low cost.

The vast majority of the carrier materials used in the food industry are biomolecules. Carrier materials should be able to maintain core material and capsule properties during processing and storage, as well as being natural. They must also be stable, do not react with the core material, and should have low viscosity even at high concentrations. Polysaccharides are the most commonly used substances in foodstuffs within the carrier materials. Polysaccharides can be grouped as marine extracts (carrageenan and alginate), starch and derivatives (amylose, amylopectin, dextrins, maltodextrins, polydextrose, cellulose and derivatives), plant extracts (Arabic gum, karaya gum, galactomannans, pectins, soluble soy polysaccharides), microbial and animal origin (Dextran, chitosan, xanthan and gellan gum). Apart from natural and modified polysaccharides, protein and lipid-based materials are also widely used. Examples of milk-derived proteins include casein and gelatin. Examples of oil based materials are; Fatty acids, fatty acid alcohols, waxes (wax, carnauba, kandelila wax), glycerides and phospholipids. In addition to all these substances, PVP, paraffin, shellac and inorganic materials can also be used as carriers (Fuchs et al., 2006; Nedovic et al., 2011).

1.6.2 Encapsulation Methods

There are different encapsulation methods used in industry. These methods can be divided into 3 main categories as given in Table 1.10.

Table 1.10 Encapsulation methods (Singh et al., 2010).

Encapsulation methods	Phsyical methods	Pan coating
		Air-suspension coating
		Centrifugal extrusion
		Vibrational nozzle
		Spray-drying
	Physicochemical methods	Ionotropic gelation
		Coacervation-phase seperation
	Chemical methods	Interfacial polycondensation
		Interfacial cross-linking
		In situ polymerization
		Matrix polymerization

Pan coating method: It is the oldest industrial method and commonly used in pharmaceutical industry. By this method small and coated particles or tablets can be produced. Particles that will be coated are looped in pan and at the same time coating material is added slowly.

Air suspension method: In the air suspension method, solid core material is suspended by air that is vertically current and sprayed with solution of wall material. When the solvent is evaporated, layer of the encapsulated material is deposited onto core material. The process is repeated till achieving the desired thickness of film. Generally large size of core particle is used for this method.

Centrifugal extrusion method: In this method, liquid can be encapsulated by using a rotated extrusion head with concentric nozzles. Core material in the liquid form is pumping through the inner orifice and wall material in the liquid form.

Spray drying method is serving microencapsulation technique when the active material dissolves or suspends in a melt or polymer solution and results with trapping in dried particle. Spray drying has advantage that labile materials can easily handle. Since the interaction between the temperature and the material is kept in very short time. Moreover, the operation is very economical according to other microencapsulation methods. (Vidhyalakshmi et al., 2009).Spray drying is most effective method of encapsulation (Burgain, et al., 2011). This method has also been used for probiotic encapsulation (Burgain et al., 2011, Salar-Behzadi, et al., 2013, Ozyurt and Ötles, 2014).

Ionotropic gelation is a method occurs when the units of uric acid chains of the alginate polymers is crosslinking by multivalent cations. Cations may be zinc, calcium, aluminum or iron.

Coacervation -phase separation is consisting three consecutive steps with continuous agitaton. The first step is the formation of liquid manufacture vehicle phase, core material phase and coating material phase. The second step is deposition of coating material. The last step is the rigidization of coating material.

Interfacial polycondensation is the method that have the basis of Schotten-Baumann reaction. Which refers to the meeting of two reactants in polycondensation at the interface and react. In the reaction occurs between an acid chloride and a compound that contains an active hydrogen atom. By the proper conditions, a thin and flexible wall can form at the interface. Then, with the addition of solutions, condensed polymer walls form emulsion droplets at the interface.

Interfacial cross-linking is reproduced from interfacial polycondensation method. The aim of this method is to avoid usage of toxic solutions that used in interfacial polycondensation methods. In the method, small bifunctional monomer that is containing active hydrogen atoms is replaced by biosourced polymer. When the reaction occurs at the interface of an emulsion, the acid chloride is reacting with various functional groups of the protein, leading to the formation of a membrane.

In situ polymerization method is very similar method to interfacial polymerization. The difference of this method is that there is no reactants used in the core material. All polymerization is happening in continuous phase.

Matrix polymerization method is including the embedding of core material in a polymeric matrix while formation of the particles.

In a number of processes, a core material is imbedded in a polymeric matrix during formation of the particles. A simple method of this type is spray-drying, in which the particle is formed by evaporation of the solvent from the matrix material. However, the solidification of the matrix also can be caused by a chemical change.

1.7. Whey

Whey is a by-product of cheese production and contains about 85% of the total volume (de Wit, 1998, Madureira et al., 2007). Whey has a rich content in terms of protein, basic amino acid, lactose, salt and fat. Component of whey and their benefits are listed in the Table 1.11. For this reason it has an important place in the food industry (Siso, 1996, de Wit, 1998, Madureira et al., 2007). The effects of whey proteins and amino acids on human health have been investigated in some studies. Biological and physiological changes due to the consumption of amino acids and whey protein, such as measurement of muscle glycogen level and performance change, have been made primarily on mice and have been found to have effects on some diseases in humans thanks to advanced technology (Boza et al., 2000; Morifuji et al., 2005).

Table 1.11 Component of Whey and Their Benefits (Marshall, K. 2004)

Component	**Amount**	**Benefits**
β-Lactoglobulin	50-55 %	Essential and branched chain amino acids source
α-Lactalbumin	20-25 %	Essential and branched chain amino acids source Primary protein found in human breast milk
Immunoglobulins	10-15%	Immune modulator Primary protein found in colostrum
Lactoferrin	1-2%	Antioxidant Antibacterial, antiviral , and antifungal Promoting growth of beneficial bacteria Naturally found in breast milk, tears, saliva, bile, blood, and mucus
Lactoperoxidase	0.50%	Inhibiting growth of bacteria
Bovine Serum Albumin	5-10%	Essential amino acids source Large protein
Glycomacropeptide	10-15%	Branched chain amino acids source Lacking the aromatic amino acids phenylalanine, tryptophan, and tyrosine

Whey juice shows antioxidant and antifungal properties because of the presence of lactoferrin and lactoferricin. With this feature, it prevents the performance deterioration due to the storage of reactive oxidizers that occur during exercise in the human body (Ha et al., 2003; Cribb, 2005). In another study, fermented dairy products were found to decrease blood pressure and prevent hypertension without using harmful drugs. Bioactive peptides derived from whey protein have protective effect against hypertension by inhibiting the action of angiotensin converting enzyme (Rehberger, 2006). In addition, proteins in whey have protective effects against some types of cancer (Yerlikaya et al., 2010).

The components contained in whey are separated from whey by physical or chemical separation techniques such as precipitation, filtration, dialysis or ion exchange (ADPI, 2002). Using these techniques, the protein found in whey is separated in certain quantities. There are different varieties of whey in the market. These are enzymatically obtained whey isolates (> 90% protein), whey concentrates (25-89% protein) and whey hydrolysates (80-90%) (Manninen, 2009).

Whey is generally used as an additional protein. It is also used in yogurt and puddings to increase the gelatinization of products, in sausage and meat products due to its water binding property and in the production stages of products such as ice cream, mayonnaise and margarine as emulsifier. After the whey powder was added to the yogurt at different ratios, the behavior of *Lactobacillus acidophilus, Streptococcus thermophilus* and Bifidobacteria were examined during their shelf life and eventually they were found to be at a certain level even after shelf life (Dave and Shah, 1998). In another study, to evaluate the tolerance of *Lactobacillus casei* and *Bifidobacterium infantis* in the stomach passage, whey isolate was added to the yogurt, and the growth and viability of the probiotics increased (Doherty et al., 2010).

Yogurt and fermented dairy products are known to be rich in beneficial bacteria. It has been determined in an investigation that probiotics increase their function when they reach the digestive tract together with culture bacteria (Gerez et al., 2012). However, yogurt alone cannot serve as a protection for probiotics. Thus, the encapsulation method can be used for the attachment of probiotics so that the viability rates of the probiotics during and after the process can be increased (Sultana et al., 2000; Krasaekoopt et al., 2006).

The purpose of using whey is to increase the amount of solid matter in the yogurt and improve the rheological and sensory properties (Isleten and Karagul-Yuceer, 2006, Patocka et al., 2006, Kücükcetin, 2008, Dinçoğlu and Ardıç, 2012). Whey is appreciated as a good encapsulation material thanks to its protein structure. A protein-based method can be shown as an alternative to other coatings in encapsulation (Champagne et al., 2006). It has also been found that adding whey powder at different ratios to the yogurt enhances the viability of cultures and probiotic bacteria (Akalin et al., 2007, Ummadi and Curic-Bawden, 2008, Doherty et al., 2010, 2011, Rodrigues et al., 2011, Doherty et al. , 2012).

1.8. Aim of the Study

Whey is a valuable by-product of cheese manufacture and is converted into whey powder by spray drying. Spray drying is also an efficient way of encapsulating bacteria and whey proteins are suitable for encapsulation. Therefore, in this study, it is hypothesized that co-encapsulating probiotic bacteria during the production of whey powder by spray drying is a practical and economical way of obtaining encapsulated probiotic bacteria that can be added into various food products. Whey is known to have functional properties such as buffering capacity and antifungal action. Natural yogurt obtained without using artificial additives has the problem of short shelf life due to post-acidification and surface yeast/mold growth. Thus, in this study, the probiotic *L. acidophilus* was encapsulated into whey powder/gum arabic microcapsules during spray drying and was added into plain set-type yogurt in order to prolong shelf-life by retarding post-acidification and surface yeast/mold growth during refrigeration storage.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Bacterial Strains

Three different bacterial strains were used. *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (RICH® Yogurt cultures) were provided by Torku- Panagro Meat and Dairy Food Complex, Konya, Turkey. *Lactobacillus acidophilus* was obtained as LA-5® from Chr. Hansen, Hoersholm, Denmark.

2.1.2 Growth Media and Temperature

Trypticase soy yeast extract medium (VWR) was used for *Streptococcus thermophilus* strains after sterilization at 121°C for 15 minutes. *Lactobacillus delbrueckii* ssp. *bulgaricus* strains were grown in MRS medium (VWR) that was sterilized at 121°C for 15 minutes. For *Lactobacillus acidophilus* strains MRS medium with cysteine was used. Cysteine was added to MRS medium at 0.05% and after that the medium was sterilized at 121°C for 15 minutes. Incubation temperature was 37°C for *L. bulgaricus* and *L. acidophilus* and 45°C for *S. thermophilus*. Incubation time was 48 hours for *L. bulgaricus* and *S. thermophilus* and 72 hours for *L. acidophilus*.

2.1.3 Chemicals & Media

The chemicals and media used are listed with suppliers in Appendix A.

2.2 Methods

2.2.1 Cultivation

Yogurt starter cultures which were obtained from Panagro Meat and Dairy Food Complex were stored in 50% glycerol at -80°C. Cultures were reactivated in milk. The milk was heat treated (85°C /5min) and cooled down to 42±1°C. After filtration of milk cultures were added (1 % v/v) and put into shaking incubator at 37°C for 24h.

Lactobacillus acidophilus was stored in glycerol at -80°C. Probiotic bacteria were reactivated in MRS Broth (15mL) at 37°C incubating for 48h. In order to reach high number of cells, three tubes of MRS Broth were combined and centrifuged at 9000 rpm and 4°C for 10 min. 35 mL of MRS Broth was drained and cell concentrate was obtained in 10 mL MRS Broth.

2.2.2 Spray Drying

Spray drying was carried out by using a spray dryer (Labplant® Spray Dryer SD-06A) in Middle East Technical University, general laboratory. The nozzle (0.5 mm diameter) used was able to form liquid droplets. Spray drying process was carried out in the same manner as described by Burgain, et al. (2011). Schematic presentation of spray dryer used is given in the Figure 2.1.

The solution with the help of plastic pipe and pump is pressurized and then atomized with nozzle to form powder into drying chamber. The hot air is given from above of drying chamber. Hot air flowing at speed 4.3 m/s makes available to evaporate the solvent. The microcapsules are transported to cyclone separator for recovery (Burgain, et al., 2011).

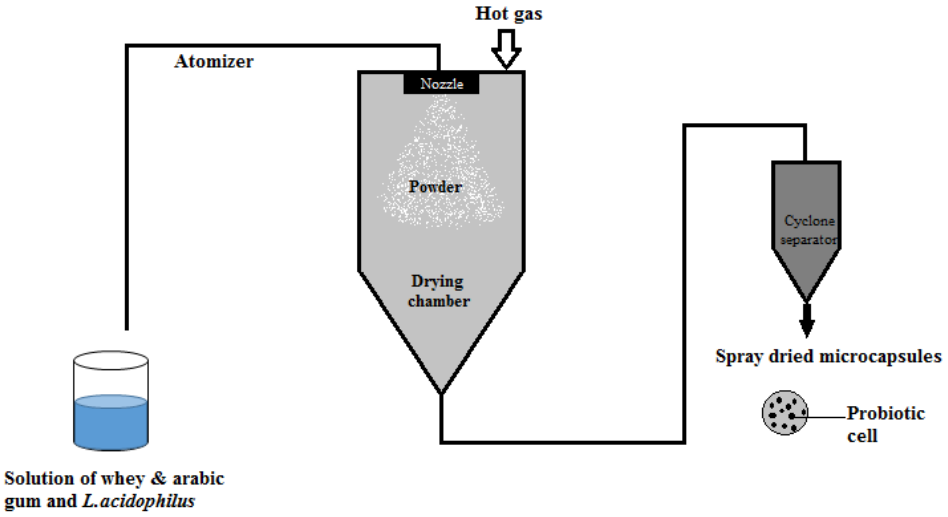

Figure 2.1 Schematic presentation of spray drying process.

2.2.2.1 Optimization of Spray Drying

For the optimization, the independent variables were inlet temperature of air (T_{inlet}), pump rate of spray drier (P) and ratio of whey to arabic gum of coating material (R). Experiments were designed using a central composite design with 2 replicates in the centre and 30 runs were done. Independent variables and their levels are listed in Table 2.1. Optimization was done by applying response surface methodology (RSM) with central composite design. Design of experiments and statistical analysis were carried out by using Minitab trial version 18 (Minitab, Ltd. United Kingdom).

Table 2.1 Independent variables used in the optimization of spray drying conditions

	Codes and values of independent variables of design using RSM		
Levels	-1	0	+1
Inlet Temperature (T_{inlet}, °C)	100	120	140
Pump Rate (P,rpm)	10	18	25
Ratio of whey to arabic gum (R)	1:1	3:1	1:0

For every trial, 200 mL aqueous solution was prepared with 20% of dry matter with the ratio of whey to arabic gum specific for that trial. The prepared emulsion was homogenized at 10000 rpm for 2 min. The probiotic *L. acidophilus* LA-05 (2.5 % v/v) was added to emulsion and stirred under sterile conditions. The emulsion was loaded into spray drier and product was obtained.

Responses were selected as production efficiency and encapsulation efficiency of powder. Production efficiency as percentage was calculated with the following equation:

$$Production\ efficiency\ (\%) = \frac{Sample\ weight\ (g)}{Dry\ matter\ of\ emulsion\ (g)} \times 100 \qquad (2.1)$$

Dry matter of emulsion was fixed at 20% for 100 mL of emulsion, therefore for 200 mL of emulsion it was taken as 40 in all experiments.

Since enumeration of free *L. acidophilus* was found as CFU/mL, it was converted to CFU/g to calculate encapsulation efficiency with the following equation (2.2):

$$Number\ of\ cells\ (CFU/g) = \frac{Number\ of\ cells\left(\frac{CFU}{mL}\right) \times A\ (mL)}{D(g)} \times E \qquad (2.2)$$

Where A is amount of cells in broth (10mL), D is dry matter amount of emulsion (40 g) and E is maximum production efficiency.

Encapsulation efficiency as percentage was calculated according to following equation:

$$Encapsulation\ efficiency(\%) = \frac{Number\ of\ probotic\ cells\ after\ spray\ drying\ (\log(\frac{Cfu}{g}))}{Number\ of\ probotic\ cells\ before\ spray\ drying\ (\log(\frac{Cfu}{g}))} \times 100$$

(2.3)

2.2.2.1.1 Response Surface Methodology

Response surface methodology was used with a central composite design to compose experiments and model the process. ANOVA was used with the regression models given below equation for statistical analysis.

$$Y = \beta_0 + \sum_{i=1} \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j \quad (2.4)$$

where: Y is the predicted response of the dependent variable, β_0 is the second order reaction constant, X_i and X_j are the independent variables, β_i is the linear regression coefficient, β_{ii} is the quadratic regression coefficient and β_{ij} is the regression coefficient of interactions between two independent variables (Rouissi et al.,2013).

The effects of independent variables were analyzed with respect to polynomial model given in equation 2.4. Where independent variables are inlet temperature of air, pump rate of spray drier, and ratio of whey to arabic gum of coating material. Probability (p) values and the student's test were used to evaluate the statistical significance of the variables studied and interaction between them.

2.2.2.2 Production of Microcapsules

The probiotic microorganism was encapsulated by spray drying method as described as Burgain, et al. in 2011. Whey and Arabic gum solution was used as wall and coating material. An aqueous solution was prepared according to optimized ratio with 20% of dry matter. The emulsion was homogenized at 10000 rpm for 2 min (Ultraturrax homogenizer, IKA Works Inc.,Staufen, Germany). The cell concentrate of *L. acidophilus* LA-05 (2.5 % v/v) was added and stirred under sterile conditions.

Final emulsion was loaded into spray drier and powder of microencapsulated *L. acidophilus* LA-05 in whey & arabic gum was obtained.

2.2.2.2.1 Enumeration of *L. acidophilus* in Microcapsules

Viability of microencapsulated LA05 was determined. 1 g of microencapsulated powder was loaded into 0.8 % (w/v) saline solution and serial dilutions were made. Then spread plating was done on 1 % Sorbitol / MRS agar using petri plates. MRS agar was prepared according to manufacturer's directions. After that MRS Agar and sorbitol solution were autoclaved at 121°C for 15 min, respectively. Just before pouring into petri dishes, 10 mL sorbitol solution was added for 90 mL MRS agar. Therefore 1 % final concentration was achieved (Gebara et al., 2015).

The plates were incubated at 37°C for 72 hours in an anaerobic jar using GENbox Anaerob with subsequent enumeration of the probiotic microorganisms.

Efficiency was calculated as given in equations 2.1 and 2.3.

2.2.2.2.2 Viability of *L. acidophilus* in Microcapsules Exposed to Simulated Gastrointestinal Tract Conditions

In order to determine viability of probiotic microorganism during passage through the gastrointestinal tract, simulated gastric juice and intestinal juice were prepared according to Valero-Cases and Frutos (2015). Simulated gastric juice (SGJ) at pH 3.0 and simulated intestinal juice (SIJ) at pH 7.0 were used in experiment.

In order to prepare simulated gastric juice pepsin (3g/L) was added to MRS broth. The pH was adjusted to 3 with 0.1N HCl. Solution was homogenized for 2 min in a vortex and then sterilize-filtered through a membrane (0.45µm). The microcapsules (1 g) were added to 9 mL of SGJ and solution was homogenized for 2 min in a vortex. SGJ was incubated during 60 min at 37°C. The enzymatic reaction was stopped by neutralization with 1 N NaOH to pH 7.

Simulated intestinal juice was prepared with 4.5 g/L bile salts in distilled water. The pH of MRS broth was adjusted to 7 with NaOH 0.1 N. Both solutions were sterile-filtered through a membrane (0.45μm). 9 mL of SIJ and MRS Broth were added to suspension of SGJ up to volume 20 mL and was incubated at 37°C for 60 min.

The viable count of SGJ and SIJ was accomplished by the plate count method in MRS-Sorbitol agar and expressed as log CFU g^{-1}.

Efficiency was calculated as given in equations 2.1 and 2.3.

2.2.2.2.3 Particle Size Analysis of Microcapsules

Particle size distribution of microcapsules was determined by wet dispersion module (Hydro 2000S) of a particle size analyzer (Malvern, Mastersizer 2000SR). The Hydro 2000S module is equipped with a stirrer and the speed of stirrer was fixed to 2000 rpm and 15 s. Particle size distributions were summarized by the characteristic volume-based D10 value representing 10 %, D50 value representing 50 % and D90 value representing 90 %, of the total micro particle population (Ozdemir et al., 2015).

2.2.3 Yogurt Production

For production of set yogurt, Torku whole dairy milk was used. The milk was heated up to 85°C and kept at that temperature for 5 min. After that milk was cooled down to 42±1°C. Treated milk was divided into eight portions (150 mL) .In order to compare effect of WP and P-WP/AG, four different samples were prepared with two parallels. The compositions of yogurt samples are given in the Table 2.2. Fermentation of yogurts were followed up at 42±1°C for 12h. After 12h pH was determined and yogurts were stored at refrigerator (4±1°C).

Table 2.2 Compositions of Yogurt Samples

Yogurt sample	Composition
1	Milk + 2.5% (v/v) yogurt starter cultures
2	Milk + 2.5% (v/v) yogurt starter cultures + 2% (w/v) WP/AG
3	Milk + 2.5% (v/v) yogurt starter cultures + 2% (w/v) WP/AG + 1% (v/v) *Lactobacillus acidophilus* free
4	Milk + 2.5% (v/v) yogurt starter cultures + 2% (w/v) P-WP/AG

2.2.3.2 Microbiological Characterization of Yogurt Samples

Microbiological analysis included *L. acidophilus, S. thermophilus, L. bulgaricus*, total bacteria, psychotropic bacteria and yeast & mold count as CFU/g. 1 g of yogurt was diluted in 0.8 % (w/v) saline solution and for releasing microorganisms it was suspended to vortex for 2 min. After serial dilution was made, samples were spread plated as 0.1 mL in petri dishes (Gebara et al., 2015).

The enumeration was done with selective methodologies.

2.2.3.2.1 *Lactobacillus acidophilus* Enumeration

In order to enumerate *Lactobacillus acidophilus*, MRS agar with the addition of 0.05 % cysteine was prepared according to manufacturer's directions. MRS Agar was sterilized at 121°C for 15 minutes and poured into petri dishes. Incubation was done at 37°C for 3 days (DSMZ, 2012; Lima et al., 2008; Ashraf and Shah, 2011).

2.2.3.2.2 *Streptococcus thermophilus* Enumeration

For enumeration of *Streptococcus thermophilus* trypticase soy agar with the addition of yeast extract (10 %) was prepared according to manufacturer's directions. Trypticase soy yeast extract medium was sterilized at 121°C for 15 minutes and after poured into petri dishes. Incubation was done at 45°C for 2 days (DSMZ, 2012).

2.2.3.2.3 *Lactobacillus bulgaricus* Enumeration

For enumeration of *Lactobacillus bulgaricus* MRS agar was prepared according to manufacturer's directions. MRS agar was sterilized at 121°C for 15 minutes and after poured into petri dishes. Incubation was done at 37°C for 2 days (DSMZ, 2012; Lima et al., 2008; Ashraf and Shah, 2011).

2.2.3.2.4 Total Bacteria Enumeration

For enumeration of total bacteria plate count agar was used - PCA Agar was prepared according to manufacturer's directions. PCA Agar was sterilized at 121°C for 15 minutes and poured into petri dishes. Incubation was done at 35°C for 3 days (Nyambane et al., 2014).

2.2.3.2.5 Psychrotropic Bacteria Enumeration

Enumerating psychrotropic bacteria was done with the usage of plate count agar - PCA Agar was prepared according to manufacturer's directions and sterilized at 121°C for 15 minutes. After that PDA Agar was poured into petri dishes. Incubation was done at 4°C for 10 days (Salustiano et al., 2003).

2.2.3.2.6 Mold and Yeast Enumeration

Mold and yeast enumeration was done on potato dextrose agar - PDA Agar was prepared according to manufacturer's directions. It was sterilized at 121°C for 15 minutes and after poured into petri dishes. Incubation was done at 25°C for 5 days (Ozdemir and Vural, 2016).

2.2.3.3 Sensory Analysis

The sensory study was done by professors, instructors and laboratory members at Konya Food and Agriculture University Strategic Products Research and Development Center (SARGEM) Special Food Control Laboratories. Test for acceptance of yogurts was conducted with 7 participants.

Yogurts containing four different composition which are (1) control yogurt, (2) control yogurt with whey and arabic gum, (3) free *L. acidophilus* yogurt and (4) encapsulated powder yogurt was used for sensory analysis. Each participant taste yogurts and they are requested to evaluate yogurt 1 to 5, where 1= dislike extremely, 5=like extremely. Evaluation was done with the following attributes of yogurts: taste, smell, appearance, texture and general acceptability.

2.2.3.4 Statistical Analysis

Different yogurt compositions (yogurt 1, 2, 3 and 4) and storage (day 0, 7, 14, 21 and 28) are the independent variables studied in this study. Minitab® 18.1.1 was used to determine if there were difference significantly between independent variables and dependent variables. Dependent variables were pH of yogurt samples, viability of *L. acidophilus, S. thermophilus, L. bulgaricus,* total bacteria, mold & yeast and psychotropic bacteria respectively. The analysis of variance (ANOVA) test was utilized by Minitab. In order to compare analysis, Tukey's Multiple Comparison Test was used with 95 % confidence interval ($p \leq 0.05$). For each variable, all results were double replicated.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Experimental Design

In this study, *L. acidophilus* was encapsulated in whey powder and was incorporated in milk for plain set-type yogurt production. Thereby, yogurt was produced with the added benefits of a probiotic culture and the functional properties of whey powder. Whey powder is shown to have antifungal effect and has buffering capacity. Due to these properties, WP is expected to prolong the shelf life of yogurt. Furthermore, probiotic set-type yogurt is not present on the market. This is due to the fact that the standard yogurt cultures *S. thermophilus* and *L. bulgaricus* have inhibitory effect on probiotic bacteria. Therefore, probiotic bacteria are added at the required number of cells (minimum 10^6 CFU/g) after yogurt is formed from milk. Thereby, stirred yogurt is produced. Nevertheless, decrease in the viability of probiotic bacteria remains to be a problem unless they are protected e.g. by encapsulation. Whey powder is a valuable by-product of the milk industry and is produced by spray drying. Spray drying is also used in encapsulation. Thus, production of probiotic encapsulated whey powder is both practical and economical, and the resulting powder can be used in many food products.

Experimental part of this study was divided into two parts. The first part was the optimization of spray drying conditions. Inlet temperature, pump rate and ratio of whey powder to arabic gum were optimized by Response Surface Methodology (RSM). Probiotic bacteria *L. acidophilus* was added to a solution of whey and arabic gum for encapsulation before loading into the spray dryer.

In the second part of the study, probiotic encapsulated whey powder was added into milk and yogurt was produced using yogurt cultures *L. bulgaricus* and *S. thermophilus*. Free cells of *L. acidophilus* and whey powder & arabic gum mixtures without probiotic were used as control yogurt. Physiochemical, microbiological and sensorial properties of samples were determined during storage at 4°C. The sensory analysis was done to determine preference of consumers.

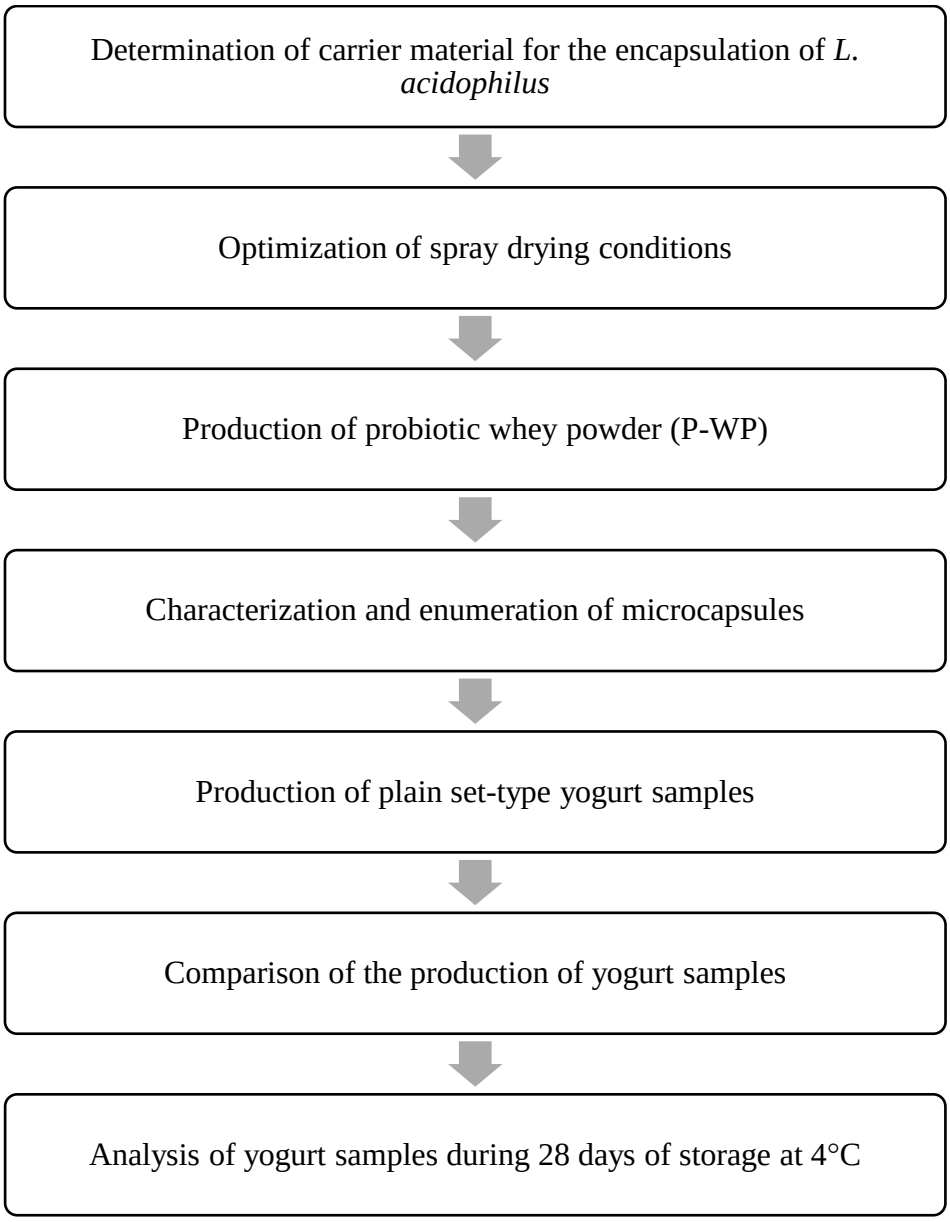

Figure 3.1 Flow chart of experimental design.

3.1.1 Determination of Carrier Material for Encapsulation

Whey is a by-product of cheese production. It is rich in protein, amino acid, salt and fat. Therefore, whey powder is produced by spray drying and is added into several food products. Moreover, it has buffering capacity, which is slowing down the acid formation in fermented products. Whey protein isolate was studied as encapsulation coating material in some researches. Nevertheless, whey powder formed without purifying of proteins has not been used in encapsulation in spray drying at all. In this study, it was decided to use whey as main carrier material. However, whey was not found to be efficient for encapsulation on its own. For better encapsulation, different polymer types were studied to develop a formulation for encapsulation. Locust bean gum, arabic gum, alginate and maltodextrin were examined and added to whey solution at different ratios. Alginate was examined since it is the most widely used material in encapsulation (Krasaekoopt et al., 2004). However, in the study of Burgain et al., 2011, alginate beads were reported to be sensitive to the acidic environment, which is not proper for fermented dairy product. When locust bean gum was studied, powder could not be obtained by spray drying because of over gelatinization. Yet, arabic gum was found to form smooth spherical micro particles during spray drying (Burgain et al., 2011). Likewise in our studies, arabic gum was found to be the most efficient secondary carrier material for encapsulation in whey powder based on yield and structure.

Since recycling of whey is within the objective of this study, whey powder rate to other carrier material was held higher. Namely, in the optimization process, whey powder was used at least at 50 % (1:1) in the mixture of carrier material.

3.2 Results of Optimization

The results of optimization are presented in Table 3.1. Thirty different experiment were done with spray dryer with different parameters. The independent variables are inlet temperature (100,120 and 140°C), pump rate (10, 18 and 25 rpm) and ratio of whey to arabic gum (50%; 1:1, 75%; 3:1 and 100%; 1:0). The dependent variables are encapsulation efficiency and production efficiency. These parameters were modeled by Response Surface Methodology and results were calculated as described in section 2.2.2.1.

The optimum conditions for the production of P-WP/AG were set by the help of the response optimizer tool Minitab® 18.1.1 by ANOVA. Accordingly, thirty sets of randomly generated experiments with different combinations were studied in spray dryer. In the Table 3.1, experimental design for production of microcapsules and studied responses are given.

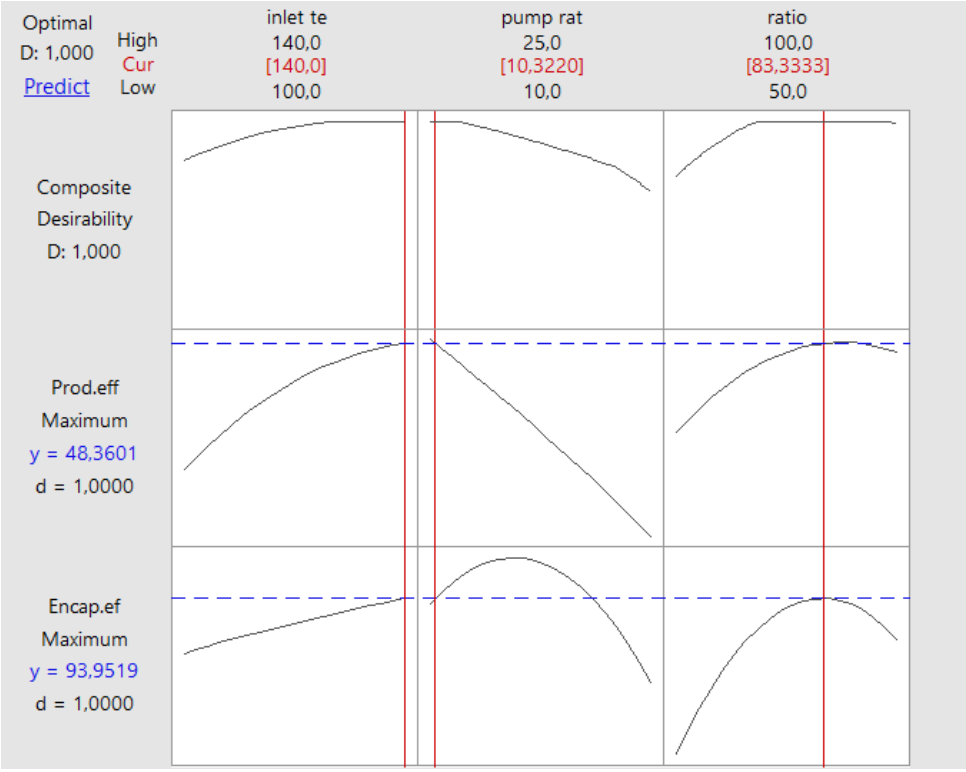

Figure 3.2 Optimization of Spray Drying Conditions for the Production of P-WP/AG.

Based on the results, optimal conditions were found of 140°C for inlet temperature, 10.32 rpm of pump rate and 0.83: 0.17 for ratio of whey to arabic gum (prepared as a 20% solution, as described in materials and methods) which resulted in 93.95% of maximum encapsulation efficiency and 48.36% of maximum production efficiency as shown in the Figure 3.2.

Encapsulation efficiency was predicted to be higher than 80% according to the literature. In this study, 93.95 % of encapsulation efficiency was reached which is much higher than the literature. However, the production efficiency was low in the optimization process. According to the literature, encapsulation production efficiency varies between 62.3% and 95.7% (Carneiro et al., 2013). The process conditions for encapsulation may affect the production efficiency, however, it is likely that this low efficiency is due to technical reasons related to the spray dryer (Labplant® Spray Dryer SD-06A). The loss in the powder during production was very high from the outlet pipe of air of the spray dryer.

In fact, in some trials, powder production could not be seen because of the conditions of that run. Namely, when the inlet temperature was low and the pump rate was high, emulsion could not be evaporated and powder formation was not observed.

Table 3.1 Experimental design and studied responses

Experiment code	Inlet Temperature (°C)	Outlet Temperature (±2°C)	Pump Rate (rpm)	Ratio of W:AG	Sample (g)	Enumeration of initial LA-5 (log CFU/g)	Enumeration of LA-5 in capsules (log CFU/g)	Encapsulation efficiency (%)	Production efficiency (%)
1	140	55	17.5	1:1	13.19	10.19	8.03	78.78	32.98
2	120	55	17.5	3:1	10.61	10.68	8.91	83.45	26.53
3	120	60	10	1:0	18.21	10.11	8.68	85.89	45.53
4	140	71	10	3:1	18.02	10.71	8.52	79.56	45.05
5	120	54	17.5	3:1	9.93	10.72	8.45	78.83	24.83
6	120	60	10	1:1	13.27	10.60	8.30	78.30	33.18
7	100	40	25	3:1	0.79	0.00	0.00	0.00	1.98
8	100	41	17.5	1:1	7.68	10.54	8.48	80.40	19.20
9	100	42	17.5	1:0	1.23	0.00	0.00	0.00	3.08
10	100	0.00	25	3:1	0.00	0.00	0.00	0.00	0.00
11	140	53	25	3:1	8.18	10.34	8.61	83.28	20.45
12	120	45	25	1:0	7.82	0.00	0.00	0.00	19.55
13	120	49	17.5	3:1	11.69	10.64	8.74	82.12	29.23
14	120	49	17.5	3:1	12.59	10.77	8.61	79.96	31.48

Table 3.1 Experimental design and studied responses (Continued)

Experiment code	Inlet Temperature (°C)	Outlet Temperature (±2°C)	Pump Rate (rpm)	Ratio of W:AG	Sample (g)	Enumeration of initial LA-5 (log CFU/g)	Enumeration of LA-5 in capsules (log CFU/g)	Encapsulation efficiency (%)	Production efficiency (%)
15	120	61	10	1:1	16.2	9.37	10.36	8.20	79.18
16	100	44	17.5	1:1	6.95	9.22	10.58	8.45	79.84
17	140	62	17.5	1:1	10.42	9.52	10.71	7.78	72.64
18	120	53	17.5	3:1	12.65	9.51	10.61	8.73	82.28
19	100	53	10	3:1	11.91	9.52	10.64	8.78	82.47
20	140	71	10	3:1	17.22	9.45	10.41	8.52	81.79
21	120	0.00	25	1:1	0.00	0.00	0.00	0.00	0.00
22	140	56	17.5	1:0	10.31	9.43	10.62	8.83	83.14
23	120	52	17.5	3:1	12.71	9.53	10.63	8.88	83.52
24	120	0.00	25	1:0	0.00	0.00	0.00	0.00	0.00
25	100	0.00	17.5	1:0	0.00	0.00	0.00	0.00	0.00
26	120	0.00	25	1:1	0.00	0.00	0.00	0.00	0.00
27	120	58	10	1:0	18.38	9.72	10.66	8.63	80.97
28	100	50	10	3:1	14.72	9.62	10.65	8.86	83.14
29	140	57	25	3:1	9.58	9.47	10.69	8.76	81.98
30	140	62	17.5	1:0	15.78	9.67	10.67	8.58	80.39

3.2 Analysis of Microcapsules

Enumeration of *L. acidophilus* was done before and after microencapsulation by taking samples either from P-WP/AG and prior to spray drying directly. *L. acidophilus* was enumerated in MRS agar by growing at 37°C incubator for 48h. After spray drying treatment, *L. acidophilus* was encapsulated in whey and arabic gum matrix. The enumeration of *L. acidophilus* was done according to method given in 2.2.2.2.1. At the end of microencapsulation, the results showed that the number of cells was decreased to 8.95 log (CFU/g) as given in the Table 3.2. The efficiency of encapsulation in terms of viability of *L. acidophilus* was calculated as 95 % which showed the good result of spray drying, as Carneiro et al. (2013) reported that encapsulation efficiency varies from 62.3% to 95.7%. The decrease in the number of cells can be explained by the exposure of high temperature during spray drying. However, the cells were not much affected because they were exposed to high temperature for a very short time in spray drying.

Table 3.2 Viability of *L. acidophilus* before and after encapsulation.

	Before encapsulation	**After encapsulation**	**Yield (%)**
Number of *L. acidophilus* cells (log(CFU/g))	9.42	8.95	95

3.3 Viability of Free and Encapsulated LA in Simulated Gastrointestinal Tract

According to Fuller, 1989 and Awaisheh, 2012, probiotics can provide microbial balance and improve health of host when they are taken in adequate amount of cells. Moreover, they can show their efficiency in human intestine. Therefore, probiotics should survive inBB food during storage and after consumption.

Simulated gastrointestinal tract study was important for these reasons. In this study, free and encapsulated *Lactobacillus acidophilus* was studied under simulated gastrointestinal tract conditions according to the method given in 2.2.2.2.2. The results of experiment is given in Table 3.3. When the yield was calculated, it was seen that encapsulated *L. acidophilus* can survive better than free *L. acidophilus* under simulated gastrointestinal tract. The yield of free *L. acidophilus* was calculated as 75.47 % and the yield of encapsulated *L. acidophilus* was calculated as 89.16 %. In the study of Burgain et al., 2011, whey proteins were found to be efficient in encapsulation in terms of delivering viable cells to the GI tract when added to dairy products. In addition to that, Vidhyalakshmi et al. (2009) proved that microencapsulation of probiotic bacteria improves the viability in acidic environment and helps to transmit viable cells to host's gastrointestinal tract. The results obtained in this study support the protective effect of encapsulation on *L. acidophilus* cells.

Table 3.3 Viability of free and encapsulated *L. acidophilus* before and after exposure to simulated gastrointestinal tract conditions

	Before exposure	**After exposure**	**Yield (%)**
Number of free *L. acidophilus* cells (log(CFU/g))	9.42	7.11	75.47
Number of encapsulated *L. acidophilus* cells (log(CFU/g))	8.95	7.98	89.16

3.4 Particle Size Distribution of Microcapsules

The powder of microcapsules were analyzed for particle size distributions. A desirable low micron particle size for the P-WP/AG for incorporation into dairy and other food products was achieved. Particle size analysis results of P-WP/AG were given as D10, D50 and D90 in the Table 3.4. According to the obtained results, D10 value of microcapsules ranged from 1.306 to 1.469 μm, D50 value ranged from 6.234 to 6.312 μm and D90 value ranged from 14.329 to 14.375 μm.

Table 3.4 Particle size distributions of P-WP/AG

P-WP/AG sample	D10 (µm)	D50 (µm)	D90 (µm)
1	1,306	6,234	14,329
2	1,389	6,28	14,314
3	1,469	6,312	14,375
Average	1,388	6,276	14,34

According to Keogh et al. (2004), particle size of powder should be lower than 20 µm for better quality. In this study, the D50 of the microcapsules was calculated as 6.276 µm as average as seen in the Table 3.4. The results of particle size distributions showed that, addition of P-WP/AG to yogurt did not affect the texture of yogurt samples.

In the Figure 3.3, the distribution profile of particle size of P-WP/AG was seen as a typical Gaussian distribution.

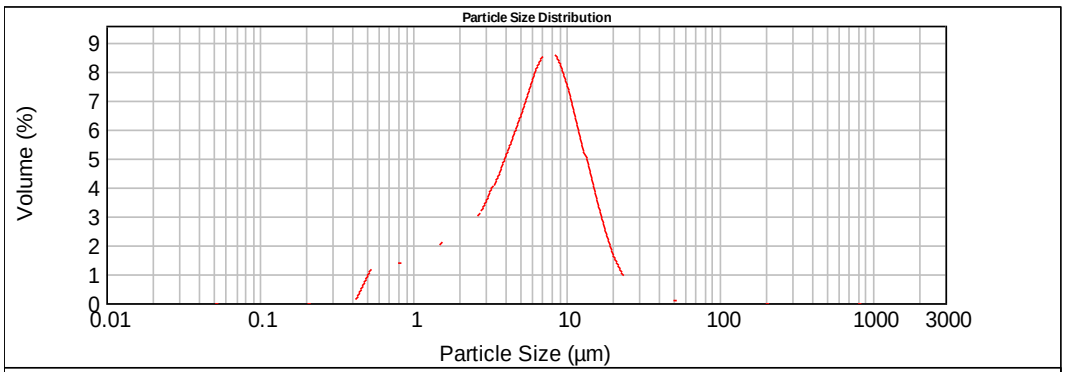

Figure 3.3 Particle size distribution profile of microcapsules

3.5 Viability of Encapsulated *L. acidophilus* in Powder During Storage at -20°C

Produced powder is recommended to be used in yogurt and also in other food materials for further studies. Therefore, viability of LA is important during storage for long time. For this purpose, P-WP/AG was produced under optimum conditions and stored at -20°C for 6 months. Enumeration of LA was done as given in the section 2.2.2.2.1.

Results showed that, the number of cells have not changed significantly. Powder comprised in the number of 10^8 CFU/g cells of probiotic even after storage for 6 months.

3.6 Production of Plain Set-Type Yogurt

In order to produce plain set-type of yogurt, milk was heated up to 85°C and then cooled down to 42±1°C. Yogurt was produced by adding of yogurt cultures to the milk. Four different yogurt compositions (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG) were studied.

Figure 3.4 Yogurt samples during incubation at 45°C.

3.7 Analysis of Yogurt Samples During Storage

3.7.1 Determination of Yogurt pH

During the storage of yogurt at 4°C, by the activity of lactic acid bacteria, acidity increases. Because of that reason acidity is the most important factor in yogurt to determine shelf life. Moreover, acidity is affecting acceptance of consumer since it gives the desired taste of yogurt. According to aim of this study, pH determination has high importance in terms of increasing shelf life of yogurt. During 28 days of analysis, four different yogurt samples were analyzed as described in section 2.2.4.1.1.

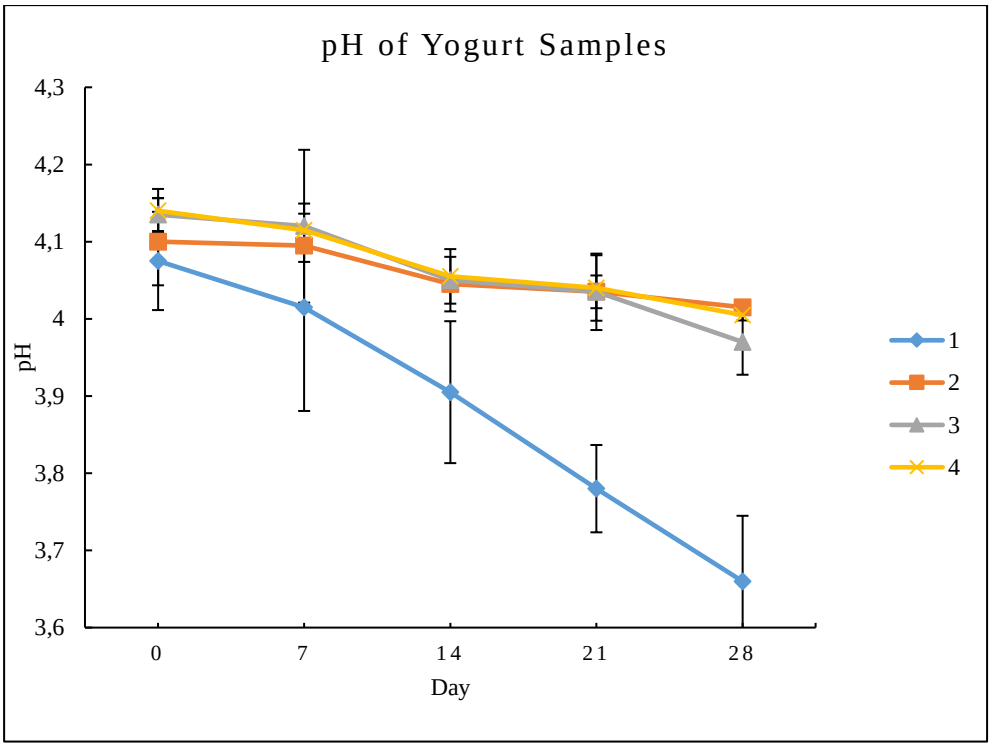

Figure 3.5 pH of yogurt samples during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG)

As seen in the Figure 3.5, yogurts including WP/AG and P-WP/AG (2, 3 and 4) showed slower decrease in pH. This result suggests the ability of WP to slow down acid formation in yogurt by its buffering capacity. Walstra et al. (2006) revealed that whey can exhibit buffering activity by the individual activities of different acid - base groups such as phosphate, lactate, amino acids and proteins. When milk is acidified, H+ ions added become bound to amino groups in the side chains of amino acids, forming NH3+ ions. With the addition of alkali, on the other hand, H+ ions are released from COOH groups, leading to the formation of COO-. Since whey have the ability to bind or release ions, any changes in pH upon addition of acid or alkali will tend to be small.In fact, addition of whey powder decreases also water activity in yogurt samples. Decreasing of water activity prevents activity of acid producer bacteria and thus post acidification slows down.

Two way ANOVA and Tukey tests were conducted with Minitab® 18.1.1 which are given in Appendix D. In addition to Figure 3.5., statistical analysis showed also that whey significantly affected the final pH values of yogurt samples. Statistics show that yogurt containing WP/AG and P-WP/AG (yogurt 2, 3 and 4) did not differ significantly, whereas yogurt, which does not contain WP (yogurt 1), is significantly different ($p \leq 0.05$).

In the study of Kailasapathy (2006), the control yogurt that included traditional yogurt cultures showed lower pH according to yogurt including encapsulated probiotic bacteria.

Since yogurt cultures are active during storage at refrigerated temperature and still produce lactic acid by fermentation of lactose, pH decreases. On the other hand, probiotic bacteria slow down acid production, when added into yogurt samples freely or in encapsulated form.

3.7.2 Microbiological Characterization of Yogurt Samples

3.7.2.1 Viability of *Lactobacillus acidophilus* During Storage

Lactobacillus acidophilus viability in yogurt during storage was monitored. The results are given in Figure 3.6. Since *L. acidophilus* was not added to first and second yogurt samples, viability of *L. acidophilus* was not determined. It was selectively grown on MRS agar with the addition of 0.05 % cysteine at 37°C for 3 days and enumerated. Results showed that the viability of encapsulated *L. acidophilus* was higher than the free form. Thus, microencapsulation provides a protective shield to *L. acidophilus* from the yogurt environment, namely low pH level of yogurt and competition with yogurt cultures. In order that a probiotic is efficient in the host's gastrointestinal tract, there should be a minimum of 10^7 to 10^9 CFU in ready-to-eat products like 200 g or mL of yogurts according to legislations (Ribeiro et al., 2014). Although 7.48×10^8 CFU/g and 5.73×10^8 CFU/g, respectively were present at the beginning of storage, the viable count of free and encapsulated probiotics was found as 6×10^5 CFU/g and 3×10^8 CFU/g, respectively at the end of storage.

Thus, encapsulation ensured this legislation requirement by protecting probiotic cells from the yogurt environment during storage.

According to statistical analysis done by Minitab Minitab® 18.1.1, there was significant difference between the cell counts of free and encapsulated *L. acidophilus* ($p \leq 0.05$).

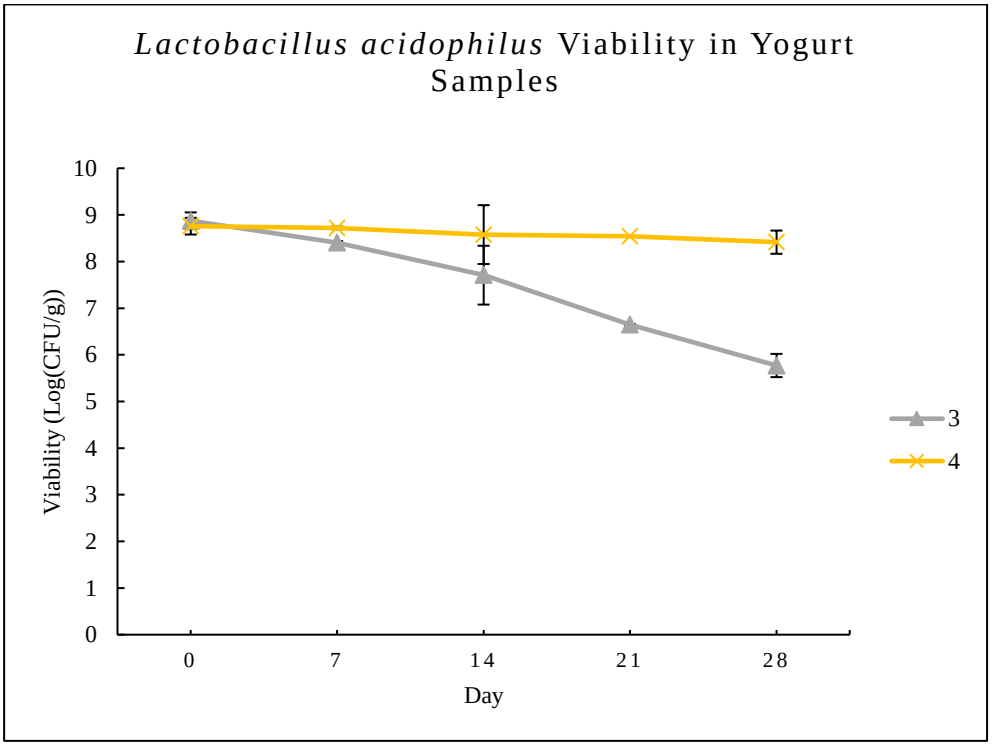

Figure 3.6 *Lactobacillus acidophilus* viability during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG)

3.7.2.2 Viability of *Streptococcus thermophilus* During Storage

S. thermophilus enumeration was done selectively in yogurt samples according to the method described in section 2.2.4.2.2 and the results are given in Figure 3.7. In yogurt fermentation, two cultures (*S. thermophilus* and *L. bulgaricus*) are grown in symbiotic relationship. This positive interaction is called proto-cooperation (Angelov et al., 2009). Although both microorganisms can grow alone in milk, this relationship maintains the yogurt texture and specific aroma.

According to the results *S. thermophilus* viability remained almost the same for 1 week of storage. By the activation of *L. bulgaricus*, acidity has increased and *S .thermophilus* cells could not survive under acidity and died after 14th day of storage. At the 14th day of storage, pH decreases significantly as seen in Figure 3.5.

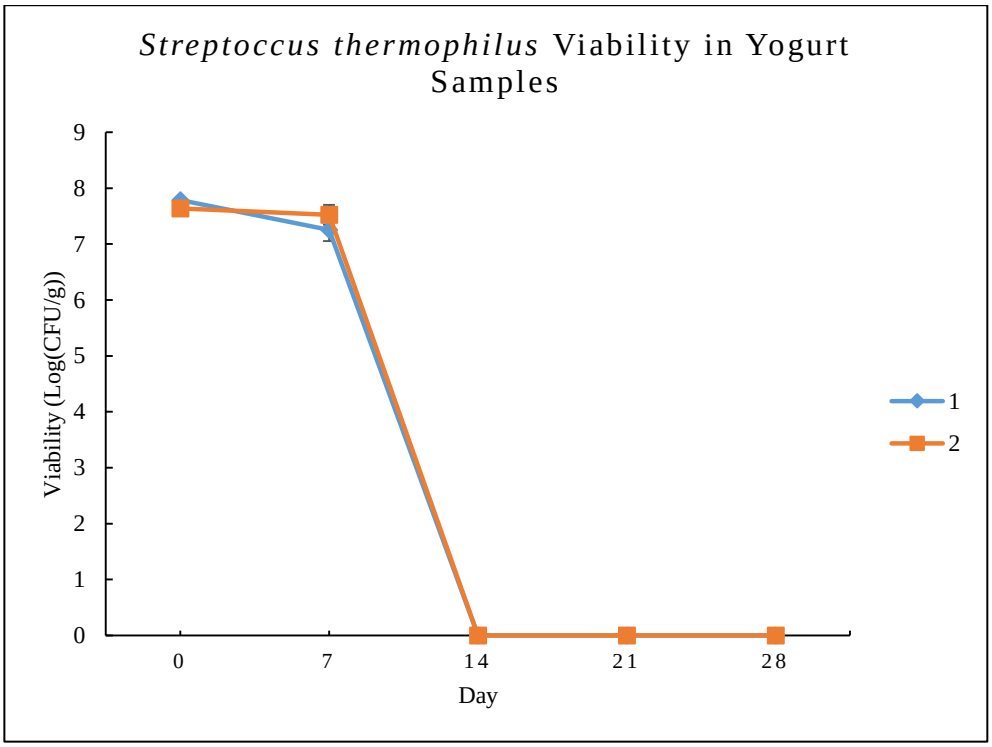

Figure 3.7 *Streptococcus thermophilus* viability during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG)

In addition to the figure, statistical analysis showed that there was not significant difference between 0 and 7 days of storage in the number of cells of *Streptococcus thermophilus*.

3.7.2.3 Viability of *Lactobacillus bulgaricus* During Storage

Lactobacillus bulgaricus enumeration was done according to the method described in the section 2.2.4.2.3. Since *L. acidophilus* can survive on MRS agar which is used for counting LB, enumeration of LB could not be done for yogurt samples 3 and 4.

The results of enumeration of *L. bulgaricus* during storage is given in Figure 3.8. LB can survive in yogurts even the end of the storage on the contrary of *S. thermophilus.* This situation showed that, although the pH of yogurts were low, *L. bulgaricus* could survive. According to Donkora (2006), *L. bulgaricus* is responsible for the post-acidification in yogurts. The significant decrease in number of cells at the end of storage can be explained by the decrease in the pH of yogurt 1.

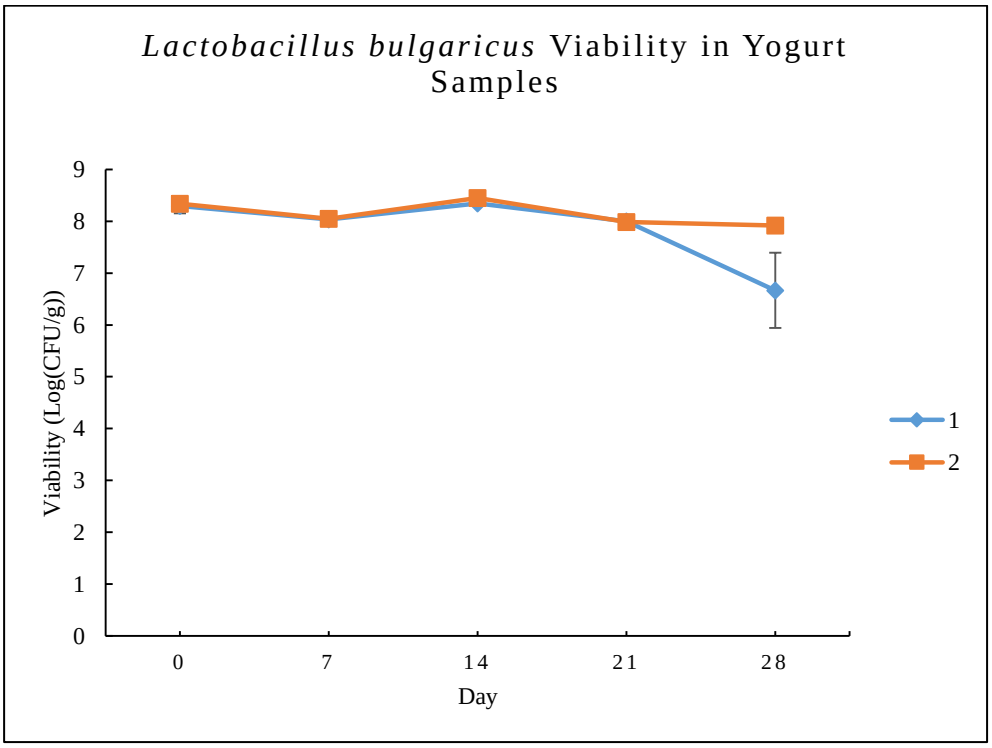

Figure 3.8 *Lactobacillus bulgaricus* viability during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG)

According to two way ANOVA and Tukey tests, a significant difference between cell counts was observed only at day 28. Accordingly, yogurt containing WP supported LB survival over long term storage.

3.7.2.4 Total Bacterial Count During Storage

Total bacterial count was done according to method given in 2.2.4.2.4. The enumeration results of total bacteria in yogurt samples is given in the Figure 3.9. Yogurt 3 (with WP/AG and free LA) has more number of cells according to other yogurt samples in the beginning. Since, it contains free *L. acidophilus* cells added. However, at the end of storage, yogurt 3 (with free LA and WP/AG) has less number of cells. This situation can be explained by the death of free cells of probiotic because of the high acidity of yogurt. At the end of storage, yogurt 4 has higher number of cells because it contains encapsulated probiotics since encapsulation prevents cells from death.

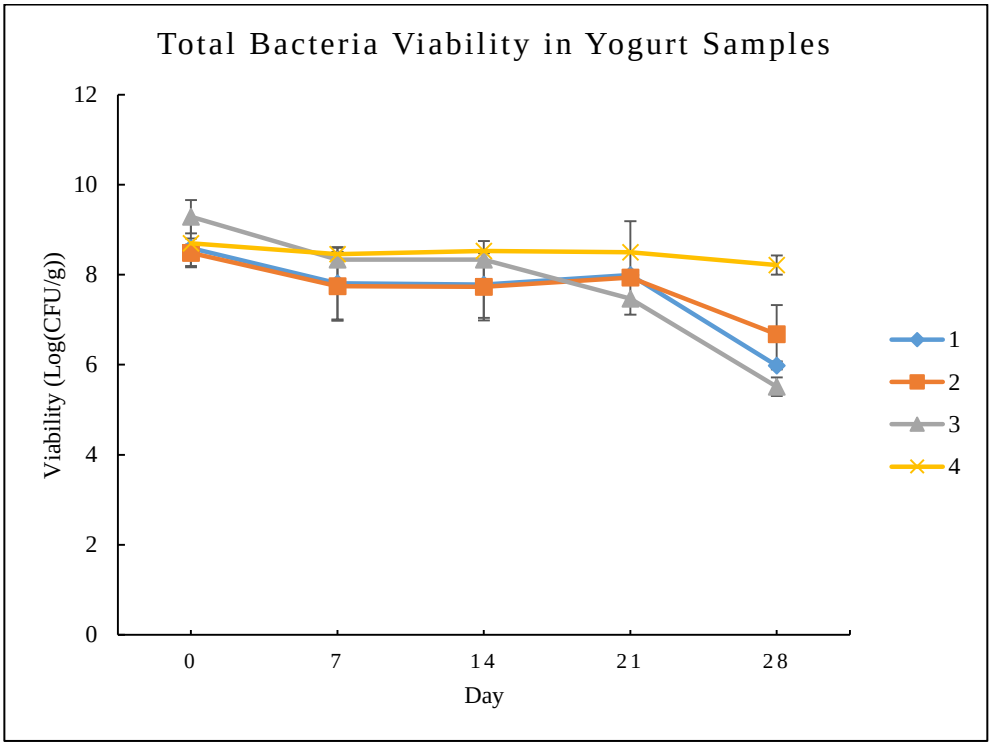

Figure 3.9 Total Bacterial count during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG)

According to the statistical analysis, there was no significantly difference between yogurts in terms of day and yogurt compositions.

3.7.2.5 Mold & Yeast Count During Storage

Mold and yeast formation on the surface of yogurts during storage has a negative effect on consumer preference. The usage of artificial additives to prevent surface mold and yeast growth is unacceptable by the consumers and is against to TS1330. In this study, mold and yeast counts were also determined. Results are given in Figure 3.10. In yogurt 4, it appears that during the process of spray drying, contamination occurred from the air used in drying. This is likely because of the lack of air filter sterilization. Although this appears to be a significant problem, it can be overcome by an instrument with filter sterilization of the inlet air. Unfortunately, in this study, such an instrument was not available.

Other bacterial activities during storage gave a different appearance to the yogurt 4 as seen in the figure 3.12.

During storage mold & yeast count in yogurt 4 were constant. In 1st yogurt (control yogurt), mold and yeast count showed a rapid increase. In yogurt 3 (with WP/AG and free LA) and yogurt 2 (control yogurt with WP/AG), mold and yeast count showed slower increase. Accordingly, WP addition to 2nd and 3rd yogurt samples was effective on mold and yeast formation. These results suggest that WP reduces the formation of mold & yeast probably by its antifungal effect because of the presence of lactoferrin and lactoferricin in it. (Madureira et al., 2007).

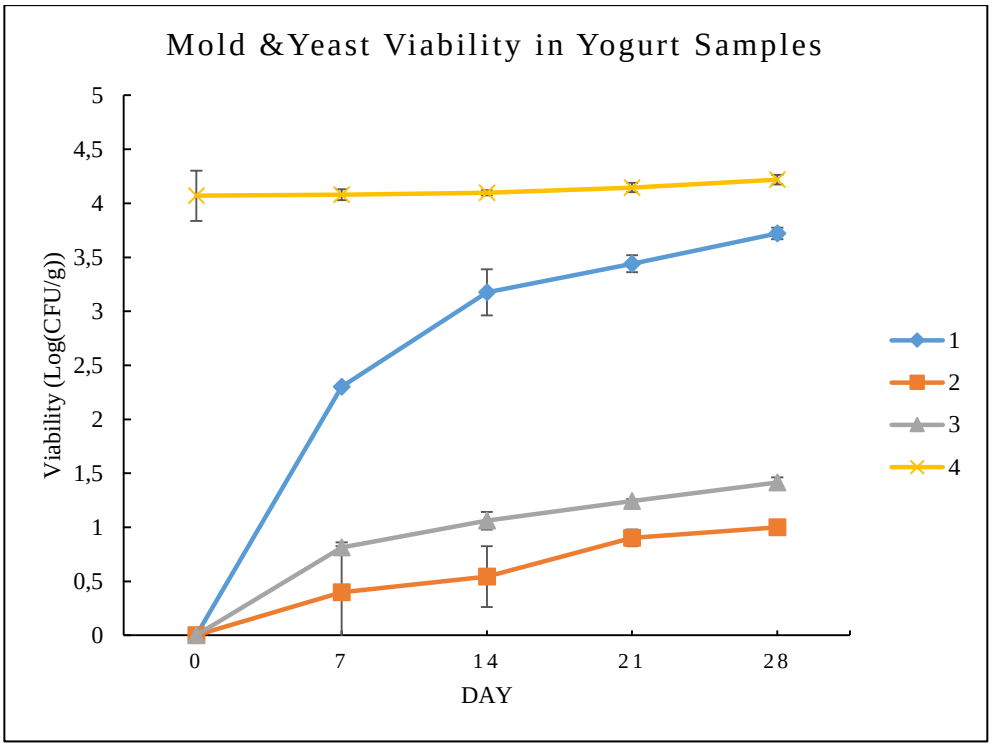

Figure 3.10 Mold & Yeast count during 28 days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG)

In Figures 3.11 and 3.12, mold and yeast formation on the surface of yogurt samples can be seen. At the end of 28 days of storage at 4°C, mold and yeast formation were seen on yogurt samples except yogurt 2. Even at the 50^{th} day of storage, no mold and yeast growth was observed in yogurt 2 which contained only whey powder and arabic gum as seen in Figure 3.13.

According to Turkish Food Codex Microbiological Criteria, mold and yeast counts lower than 10^3 CFU/g is allowed in yogurt except probiotic yogurts. In the literature, this limit is 10^5 CFU/g for probiotic yogurts (Ledenbach and Marshall, 2009). Even though yogurt containing P-WP/AG is below these limits, this can still be decreased by a proper spray drying and processing conditions.

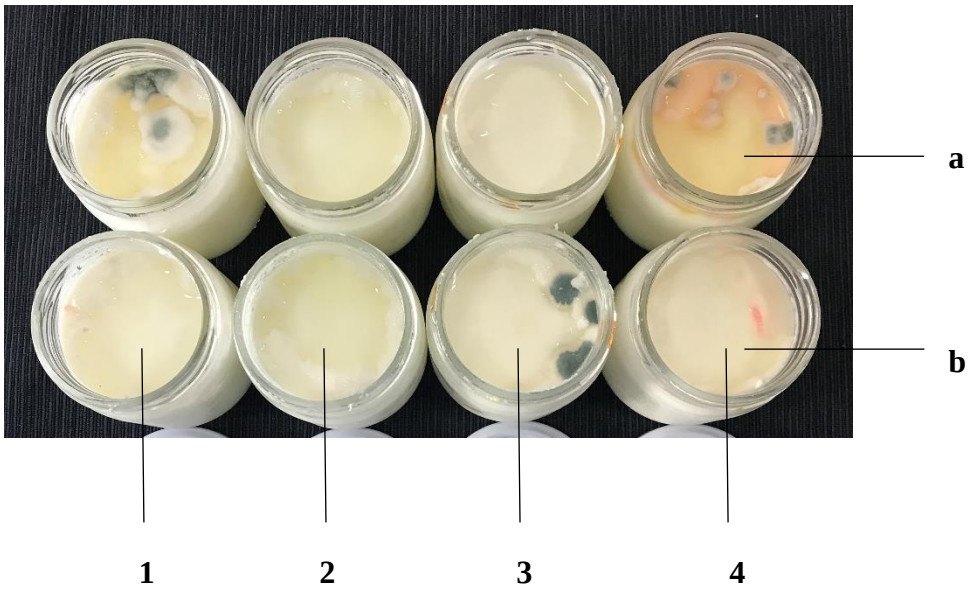

Figure 3.11 Yogurt samples after 28- days of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG and a& b are duplicates).

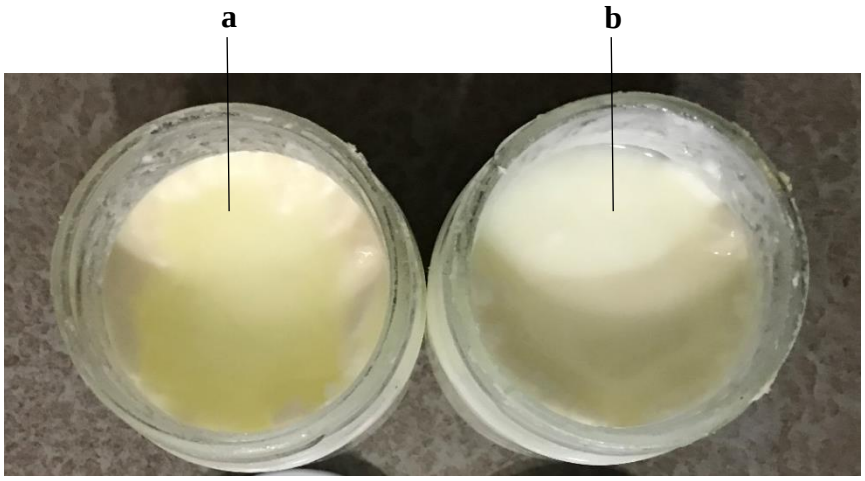

Figure 3.12 Yogurt 2 containing 2% WP/AG after 50- days of storage at 4°C.

3.7.2.6 Psychrotropic Bacteria Viability During Storage

Psychrotropic bacteria can survive under very cold environment and spoil the food when added or contaminated. Since yogurts should be kept between 2-4°C, psychrotropic bacteria are a risk for them (Ledenbach and Marshall, 2009). However, none of the samples contained psychrotropic bacteria throughout 28 days of storage at 4°C.

3.8 Sensory Analysis of Yogurt Samples

Acceptance of consumer is the most crucial parameter for food industry. Because of that, in this study sensory analysis was done to find out consumer needs. Four different yogurt samples were tasted by seven participants. Yogurts were rated according to flavor, odor, appearance, texture and general acceptability. Figure 3.13 shows the overall scores of sensory analysis of yogurt samples. The average scores show the most preferable yogurt is the first one. Yogurt 4 has slightly lower score than yogurt 1. This situation shows that addition of microcapsules did not affect adversely the sensorial characteristics of yogurt. Kailasapathy (2006) also reported that the addition of capsules including probiotics did not significantly change yogurt's properties like flavor, color, acidity or appearance. However, the addition of whey and arabic gum and addition of free *L. acidophilus* were not welcomed by panelists .Texture was affected in terms of loosing of smoothness according to same study. Consumer's choice may be affected by grittiness of yogurt because of whey. However, if the consumer is informed, sensorial properties of whey added yogurts can be desirable according to Champagne and Fustier (2007). In addition the figure, according to statistical analysis as shown in the Table 3.5 that there were no significant difference between yogurt samples by the different compositions.

Table 3.5 Means for sensory analysis of yogurts influenced by compositions of yogurts

	1.yogurt	**2.yogurt**	**3.yogurt**	**4.yogurt**
Flavor	4.14±1.07[a]	4.00±1.00[a]	4.29±0.76[a]	4.00±0.81[a]
Odor	4.00±0.58[a]	3.57±1.13[a]	3.71±0.95[a]	4.14±1.21[a]
Appearance	4.43±0.53[a]	4.29±0.49[a]	4.57±0.53[a]	4.71±0.49[a]
Texture	4.43±0.79[a]	3.86±0.90[a]	4.00±1.15[a]	4.14±0.90[a]
General acceptability	4.43±0.53[a]	4.00±0.82[a]	4.00±0.58[a]	4.29±0.49[a]

[a] Means with there is no significant difference between column.

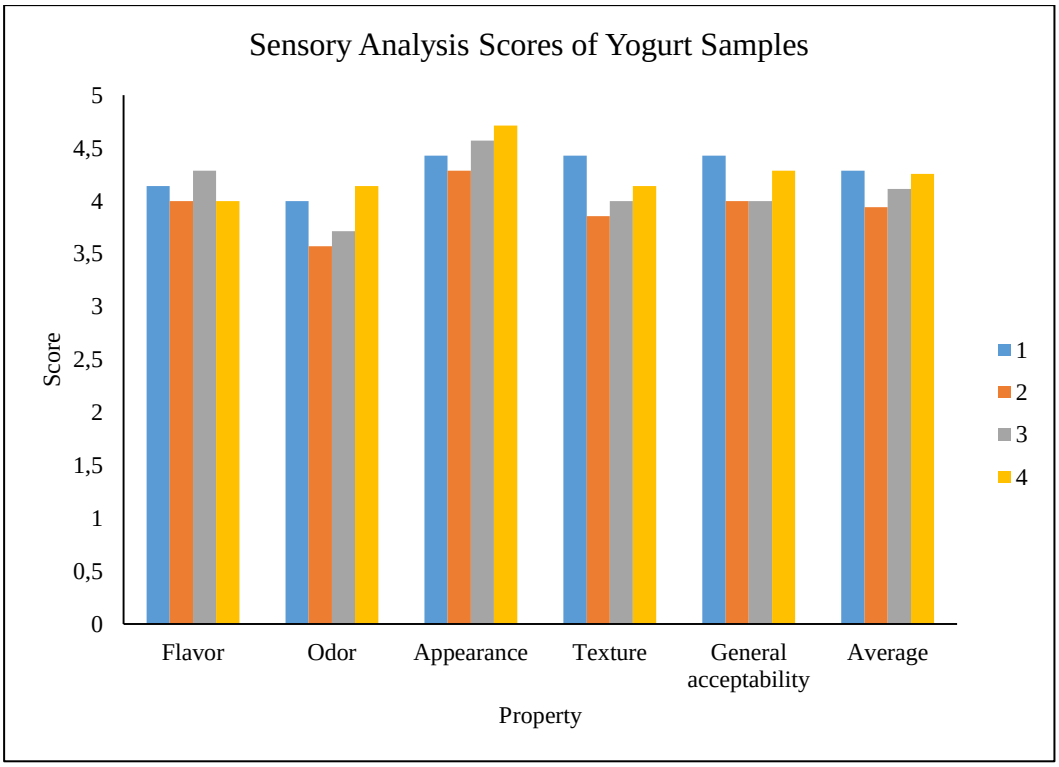

Figure 3.13 Sensory analysis scores of yogurts produced in the presence and absence of WP/AG and P-WP/AG at day 0 of storage at 4°C (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG).

CHAPTER 4

CONCLUSIONS & RECOMMENDATIONS

In this thesis, the effect of whey powder and probiotic encapsulated whey powder on the properties of yogurt, different yogurt compositions within storage at 4°C were studied in two parts. The first part included the optimization process for production of probiotic encapsulated whey powder in spray drying. The second part was including the yogurt production with different compositions (where 1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG) and determination of the effect on the properties during storage at 4°C. At the end of study, the following conclusions and outputs are obtained:

- ✓ *Lactobacillus acidophilus* can be efficiently encapsulated (with 95% encapsulation efficiency) by spray drying,
- ✓ Whey can be directly used in spray drying for encapsulation to produce smooth and proper particle sized powder without the need for whey protein isolation,
- ✓ *Lactobacillus acidophilus* can be efficiently encapsulated in whey powder containing arabic gum (with the ratio 0.83:0.17),
- ✓ *Lactobacillus acidophilus* shows high viability in microcapsules and in yogurt throughout 28 days of storage at 4°C,
- ✓ Efficiency of WP/AG on slowing down the post-acidification and formation of surface mold & yeast growth in yogurt is proved,

- ✓ Addition of WP/AG and P-WP/AG does not have a negative effect on the sensory properties of yogurt,
- ✓ Viability of encapsulated *Lactobacillus acidophilus* in WP/AG powder is not significantly affected by storage at -20°C for at least 6 months.

For further studies, production of encapsulated probiotic powder can be done by different spray dryer to increase the yield of encapsulation and contamination caused by the process conditions should be obstructed. Therefore, mold and yeast formation during storage of products can be inhibited. Moreover, this probiotic encapsulated powder can be added to other foods like chocolate, biscuits and beverages. In food industry, this probiotic encapsulated powder can be a pioneer in many innovative foods.

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APPENDIX A

CHEMICALS AND MEDIA USED IN THE EXPERIMENTS

Table A.1 Chemicals and media used in the experiments

MRS broth	VWR - C89405-522
MRS agar	VWR – C84607.0500
Tryptic soy agar	VWR - 90002-706
Yeast extract	VWR - 97063-370
Potato dextrose agar (PDA)	VWR - 90000-758
Plate count agar (PCA)	VWR - 200059-626
L- Cysteine	VWR - CA97063-474
D- Sorbitol	Sigma - S1876
Pepsin	Sigma - 77161
Bile bovine	Sigma - B3883
Sodium chloride	Sigma - 31434
Whey	Torku - Panagro
Gum arabic from acacia tree	Sigma - 30888
Sulfuric acid	Merck - 1.00713.2500
Isoamyl alcohol	VWR – 20798.295
Ammonia	Merck – 1.05432.2500
Potassium sulfate	Merck – 1.05153.1000
Copper (II) sulfate anhydrase	Merck – 1.02791.1000
Sodium hydroxide	Merck -1.06329.0500

Table A.1 Chemicals and mediums used in the experiments (Continued)

Sodium sulfate	Merck – 1.06649.1000
Hydrochloric acid	Honeywell - 10314253
Phenolphthalein	VWR-83544.180
Hydrogen peroxide	Merck – 1.08597.2500

APPENDIX B

pH OF YOGURT SAMPLES DURING 28-DAYS OF STORAGE

Table B.1 pH change of yogurt samples during 28-days of storage

Sample number	**pH**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1	a	4.03	3.92	3.84	3.74	3.72
	b	4.12	4.11	3.97	3.82	3.6
2	a	4.06	4.11	4.02	4	4.01
	b	4.14	4.08	4.07	4.07	4.02
3	a	4.12	4.19	4.05	4.02	4
	b	4.15	4.05	4.05	4.05	3.94
4	a	4.16	4.1	4.08	4.07	4.01
	b	4.12	4.13	4.03	4.01	4
1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG; a & b represent the average of double analysis of duplicates						

APPENDIX C

MICROBIOLOGICAL RESULTS OF YOGURT SAMPLES DURING 28-DAYS STORAGE

Table C.1 Microbiological results of *L. acidophilus* of yogurt samples during 28-days storage

Sample number	**Enumeration(CFU/g)**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
3	a	9×10^{8}	2.7×10^{8}	5.2×10^{7}	6×10^{6}	1×10^{6}
	b	6×10^{8}	2.4×10^{8}	5×10^{7}	3×10^{6}	2×10^{5}
4	a	7.4×10^{8}	5×10^{8}	8.6×10^{7}	3.4×10^{8}	3.6×10^{8}
	b	4.1×10^{8}	5.5×10^{8}	6.7×10^{8}	3.5×10^{8}	1.6×10^{8}
1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG; a & b represent the average of double enumeration of duplicates						

Table C.2 Microbiological results of *S. thermophilus* of yogurt samples during 28-days storage

Sample number	**Enumeration(CFU/g)**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1	a	6.5×10^7	1×10^7	0	0	0
	b	5.8×10^7	2.6×10^7	0	0	0
2	a	3.6×10^7	2×10^7	0	0	0
	b	5.2×10^7	4.6×10^7	0	0	0
1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG; a & b represent the average of double enumeration of duplicates						

Table C.3 Microbiological results of *L. bulgaricus* of yogurt samples during 28-days storage

Sample number	**Enumeration(CFU/g)**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1	a	1.5×10^8	3.3×10^8	2.2×10^8	1×10^8	9×10^6
	b	2.4×10^8	3.2×10^8	2.3×10^8	9.8×10^7	8×10^5
2	a	2×10^8	2.2×10^8	2.7×10^8	8.9×10^7	8×10^7
	b	2.3×10^8	1.6×10^8	2.9×10^8	1.1×10^8	9×10^7
1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG; a & b represent the average of double enumeration of duplicates						

Table C.4 Microbiological results of total bacteria of yogurt samples during 28-days storage

Sample number	**Enumeration(CFU/g)**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1	a	3.4×10^{8}	9×10^{6}	1×10^{7}	1×10^{8}	8×10^{5}
	b	4.4×10^{8}	1.2×10^{8}	1.1×10^{8}	9.6×10^{7}	1×10^{6}
2	a	1.6×10^{8}	9×10^{6}	9×10^{6}	8.2×10^{7}	9×10^{6}
	b	4.5×10^{8}	1×10^{8}	9.9×10^{7}	9×10^{7}	1×10^{6}
3	a	3×10^{9}	2.7×10^{8}	3×10^{8}	4.5×10^{7}	4×10^{5}
	b	9×10^{8}	1.6×10^{8}	1.6×10^{8}	1.4×10^{7}	2×10^{5}
4	a	1.6×10^{8}	2.2×10^{8}	2×10^{8}	5.7×10^{8}	2×10^{8}
	b	8.5×10^{8}	3.5×10^{8}	4.5×10^{8}	6×10^{7}	1.1×10^{8}

1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG;

a & b represent the average of double enumeration of duplicates

Table C.5 Microbiological results of mold & yeast of yogurt samples during 28-days storage

Sample number	**Enumeration(CFU/g)**					
	Duplicates	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1	a	0	200	2×10^3	2.4×10^3	4.8×10^3
	b	0	200	1×10^3	3.1×10^3	5.7×10^3
2	a	0	4	2	8.2×10^7	9
	b	0	1	5	9×10^7	11
3	a	0	6	10	18	24
	b	0	7	13	17	28
4	a	1.6×10^4	1.1×10^4	1.2×10^4	1.5×10^4	1.6×104
	b	7.5×10^3	1.3×10^4	1.3×10^4	1.3×10^4	1.8×10^4
1: control yogurt; 2: control yogurt with WP/AG; 3: yogurt with WP/AG and free LA; 4: yogurt with P-WP/AG; a & b represent the average of double enumeration of duplicates						

APPENDIX D

EFFECT OF STORAGE AND YOGURT COMPOSITION ON pH OF YOGURT SAMPLES

Table D. 1 ANOVA Table for the effect of storage and yogurt composition on pH of yogurt samples

General Linear Model: pH versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	0,21111	0,052776	16,19	0,000
Yogurt	3	0,23223	0,077411	23,74	0,000
Day*Yogurt	12	0,08956	0,007463	2,29	0,047
Error	21	0,06847	0,003260		
Total	40	0,60310			

Table D. 1 ANOVA Table for the effect of storage and yogurt composition on pH of yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4,01833	0,00895	448,84	0,000	
Day					
0	0,0942	0,0180	5,23	0,000	1,59
7	0,0621	0,0174	3,56	0,002	1,58
14	-0,0046	0,0180	-0,25	0,802	1,59
21	-0,0458	0,0180	-2,54	0,019	1,59
Yogurt					
1	-0,1313	0,0156	-8,42	0,000	1,49
2	0,0397	0,0156	2,54	0,019	1,49
3	0,0390	0,0152	2,56	0,018	1,49
Day*Yogurt					
0 1	0,0938	0,0313	3,00	0,007	2,40
0 2	-0,0522	0,0313	-1,67	0,110	2,40
0 3	-0,0165	0,0311	-0,53	0,601	2,37
7 1	0,0659	0,0309	2,13	0,045	2,35
7 2	-0,0251	0,0309	-0,81	0,426	2,35
7 3	-0,0227	0,0280	-0,81	0,425	2,15
14 1	0,0226	0,0313	0,72	0,478	2,40
14 2	-0,0084	0,0313	-0,27	0,790	2,40
14 3	-0,0028	0,0311	-0,09	0,930	2,37
21 1	-0,0612	0,0313	-1,96	0,064	2,40
21 2	0,0228	0,0313	0,73	0,473	2,40
21 3	0,0235	0,0311	0,76	0,458	2,37

Table D. 1 ANOVA Table for the effect of storage and yogurt composition on pH of yogurt samples (Continued)

Regression Equation

p = 4,01833 + 0,0942 Day_0 + 0,0621 Day_7 - 0,0046 Day_14 - 0,0458 Day_21
h - 0,1058 Day_28
- 0,1313 Yogurt_1 + 0,0397 Yogurt_2 + 0,0390 Yogurt_3 + 0,0527 Yogurt_4
+ 0,0938 Day*Yogurt_0 1 - 0,0522 Day*Yogurt_0 2 - 0,0165 Day*Yogurt_0 3
- 0,0252 Day*Yogurt_0 4 + 0,0659 Day*Yogurt_7 1 - 0,0251 Day*Yogurt_7 2
- 0,0227 Day*Yogurt_7 3 - 0,0181 Day*Yogurt_7 4 + 0,0226 Day*Yogurt_14 1
- 0,0084 Day*Yogurt_14 2 - 0,0028 Day*Yogurt_14 3 - 0,0114 Day*Yogurt_14 4
- 0,0612 Day*Yogurt_21 1 + 0,0228 Day*Yogurt_21 2 + 0,0235 Day*Yogurt_21 3
+ 0,0148 Day*Yogurt_21 4 - 0,1212 Day*Yogurt_28 1 + 0,0628 Day*Yogurt_28 2
+ 0,0185 Day*Yogurt_28 3 + 0,0398 Day*Yogurt_28 4

Fits and Diagnostics for Unusual Observations

Obs	ph	Fit	Resid	Std Resid	
5	3,9200	4,0150	-0,0950	-2,35	R
7	4,1900	4,0967	0,0933	2,00	R
25	4,1100	4,0150	0,0950	2,35	R

R Large residual

Comparisons for pH

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
0	8	4,11250	A		
7	9	4,08042	A	B	
14	8	4,01375		B	C
21	8	3,97250			C D
28	8	3,91250			D

Table D. 1 ANOVA Table for the effect of storage and yogurt composition on pH of yogurt samples (Continued)

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
4	10	4,07100	A
2	10	4,05800	A
3	11	4,05733	A
1	10	3,88700	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Day*Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping
0 4	2	4,14000	A
0 3	2	4,13500	A
7 4	2	4,11500	A B
0 2	2	4,10000	A B
7 3	3	4,09667	A B
7 2	2	4,09500	A B
0 1	2	4,07500	A B
14 4	2	4,05500	A B
14 3	2	4,05000	A B
14 2	2	4,04500	A B
21 4	2	4,04000	A B
21 2	2	4,03500	A B
21 3	2	4,03500	A B

Table D. 1 ANOVA Table for the effect of storage and yogurt composition on pH of yogurt samples (Continued)

7 1	2	4,01500	A	B		
28 2	2	4,01500	A	B		
28 4	2	4,00500	A	B	C	
28 3	2	3,97000	A	B	C	
14 1	2	3,90500		B	C	
21 1	2	3,78000			C	D
28 1	2	3,66000				D

Means that do not share a letter are significantly different.

APPENDIX E

EFFECT OF STORAGE AND YOGURT COMPOSITION ON SURVIVAL OF *L. acidophilus* IN YOGURT SAMPLES

Table E. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. acidophilus* in yogurt samples

General Linear Model: LA versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	2	3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	7,54149E+17	1,88537E+17	6,53	0,007
Yogurt	1	2,08880E+17	2,08880E+17	7,24	0,023
Day*Yogurt	4	1,84841E+17	4,62104E+16	1,60	0,248
Error	10	2,88608E+17	2,88608E+16		
Total	19	1,43648E+18			

Table E. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. acidophilus* in yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	313904000	37987365	8,26	0,000	
Day					
0	346096000	75974730	4,56	0,001	1,60
7	76096000	75974730	1,00	0,340	1,60
14	-99404000	75974730	-1,31	0,220	1,60
21	-139179000	75974730	-1,83	0,097	1,60
Yogurt					
3	-102196000	37987365	-2,69	0,023	1,00
Day*Yogurt					
0 3	189696000	75974730	2,50	0,032	1,60
7 3	-32804000	75974730	-0,43	0,675	1,60
14 3	-61304000	75974730	-0,81	0,438	1,60
21 3	-68079000	75974730	-0,90	0,391	1,60

Regression Equation

L = 313904000 + 346096000 Day_0 + 76096000 Day_7 - 99404000 Day_14
A - 139179000 Day_21
- 183609000 Day_28 - 102196000 Yogurt_3 + 102196000 Yogurt_4
+ 189696000 Day*Yogurt_0 3
- 189696000 Day*Yogurt_0 4 - 32804000 Day*Yogurt_7 3
+ 32804000 Day*Yogurt_7 4
- 61304000 Day*Yogurt_14 3 + 61304000 Day*Yogurt_14 4
- 68079000 Day*Yogurt_21 3
+ 68079000 Day*Yogurt_21 4 - 27509000 Day*Yogurt_28 3
+ 27509000 Day*Yogurt_28 4

Table E. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. acidophilus* in yogurt samples (Continued)

Fits and Diagnostics for Unusual Observations

Obs	LA	Fit	Resid	Std Resid	
6	86000000	378000000	-292000000	-2,43	R
16	670000000	378000000	292000000	2,43	R

R Large residual

Comparisons for LA

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping	
0	4	660000000	A	
7	4	390000000	A	B
14	4	214500000		B
21	4	174725000		B
28	4	130295000		B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping	
4	10	416100000	A	
3	10	211708000		B

Means that do not share a letter are significantly different.

Table E. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. acidophilus* in yogurt samples (Continued)

Tukey Pairwise Comparisons: Day*Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping	
0 3	2	747500000	A	
0 4	2	572500000	A	B
7 4	2	525000000	A	B
14 4	2	378000000	A	B
21 4	2	345000000	A	B
28 4	2	260000000	A	B
7 3	2	255000000	A	B
14 3	2	51000000		B
21 3	2	4450000		B
28 3	2	590000		B

Means that do not share a letter are significantly different.

APPENDIX F

EFFECT OF STORAGE AND YOGURT COMPOSITION ON SURVIVAL OF *S. thermophilus* IN YOGURT SAMPLES

Table F. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *S. thermophilus* in yogurt samples

General Linear Model: ST versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	2	1; 2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	8,72792E+15	2,18198E+15	36,13	0,000
Yogurt	1	1,25000E+12	1,25000E+12	0,02	0,888
Day*Yogurt	4	5,46375E+14	1,36594E+14	2,26	0,135
Error	10	6,03930E+14	6,03930E+13		
Total	19	9,87948E+15			

Table F. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *S. thermophilus* in yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	15590000	1737714	8,97	0,000	
Day					
0	36785000	3475428	10,58	0,000	1,60
7	9985000	3475428	2,87	0,017	1,60
14	-15590000	3475428	-4,49	0,001	1,60
21	-15590000	3475428	-4,49	0,001	1,60
Yogurt					
1	250000	1737714	0,14	0,888	1,00
Day*Yogurt					
0 1	8625000	3475428	2,48	0,032	1,60
7 1	-7875000	3475428	-2,27	0,047	1,60
14 1	-250000	3475428	-0,07	0,944	1,60
21 1	-250000	3475428	-0,07	0,944	1,60

Regression Equation

S = 15590000 + 36785000 Day_0 + 9985000 Day_7 - 15590000 Day_14
T - 15590000 Day_21
- 15590000 Day_28 + 250000 Yogurt_1 - 250000 Yogurt_2
+ 8625000 Day*Yogurt_0 1
- 8625000 Day*Yogurt_0 2 - 7875000 Day*Yogurt_7 1 + 7875000 Day*Yogurt_7 2
- 250000 Day*Yogurt_14 1 + 250000 Day*Yogurt_14 2 - 250000 Day*Yogurt_21 1
+ 250000 Day*Yogurt_21 2 - 250000 Day*Yogurt_28 1
+ 250000 Day*Yogurt_28 2

Table F. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *S. thermophilus* in yogurt samples (Continued)

Fits and Diagnostics for Unusual Observations

Obs	ST	Fit	Resid	Std Resid	
4	20400000	33200000	-12800000	-2,33	R
14	46000000	33200000	12800000	2,33	R

R Large residual

Comparisons for ST

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
0	4	52375000	A		
7	4	25575000		B	
14	4	0			C
21	4	0			C
28	4	0			C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
1	10	15840000	A
2	10	15340000	A

Means that do not share a letter are significantly different.

Table F. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *S. thermophilus* in yogurt samples (Continued)

Tukey Pairwise Comparisons: Day*Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping		
0 1	2	61250000	A		
0 2	2	43500000	A	B	
7 2	2	33200000	A	B	
7 1	2	17950000		B	C
14 1	2	0			C
14 2	2	0			C
28 1	2	0			C
21 2	2	0			C
21 1	2	-0			C
28 2	2	-0			C

Means that do not share a letter are significantly different.

APPENDIX G

EFFECT OF STORAGE AND YOGURT COMPOSITION ON SURVIVAL OF *L. bulgaricus* IN YOGURT SAMPLES

Table G. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. bulgaricus* in yogurt samples

General Linear Model: LB versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	2	1; 2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	1,44609E+17	3,61523E+16	54,40	0,000
Yogurt	1	9,99045E+13	9,99045E+13	0,15	0,706
Day*Yogurt	4	2,85913E+16	7,14783E+15	10,76	0,001
Error	10	6,64565E+15	6,64565E+14		
Total	19	1,79946E+17			

Table G. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. bulgaricus* in yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	171065000	5764393	29,68	0,000	
Day					
0	36185000	11528786	3,14	0,011	1,60
7	82685000	11528786	7,17	0,000	1,60
14	80935000	11528786	7,02	0,000	1,60
21	-72565000	11528786	-6,29	0,000	1,60
Yogurt					
1	-2235000	5764393	-0,39	0,706	1,00
Day*Yogurt					
0 1	-8515000	11528786	-0,74	0,477	1,60
7 1	69985000	11528786	6,07	0,000	1,60
14 1	-28265000	11528786	-2,45	0,034	1,60
21 1	3735000	11528786	0,32	0,753	1,60

Regression Equation

L = 171065000 + 36185000 Day_0 + 82685000 Day_7 + 80935000 Day_14

B - 72565000 Day_21

- 127240000 Day_28 - 2235000 Yogurt_1 + 2235000 Yogurt_2

- 8515000 Day*Yogurt_0 1

+ 8515000 Day*Yogurt_0 2 + 69985000 Day*Yogurt_7 1

- 69985000 Day*Yogurt_7 2

- 28265000 Day*Yogurt_14 1 + 28265000 Day*Yogurt_14 2

+ 3735000 Day*Yogurt_21 1

- 3735000 Day*Yogurt_21 2 - 36940000 Day*Yogurt_28 1

+ 36940000 Day*Yogurt_28 2

Table G. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. bulgaricus* in yogurt samples (Continued)

Fits and Diagnostics for Unusual Observations

Obs	LB	Fit	Resid	Std Resid	
1	152000000	196500000	-44500000	-2,44	R
11	241000000	196500000	44500000	2,44	R

R Large residual

Comparisons for LB

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
7	4	253750000	A
14	4	252000000	A
0	4	207250000	A
21	4	98500000	B
28	4	43825000	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
2	10	173300000	A
1	10	168830000	A

Means that do not share a letter are significantly different.

Table G. 1 ANOVA Table for the effect of storage and yogurt composition on survival of *L. bulgaricus* in yogurt samples (Continued)

Tukey Pairwise Comparisons: Day*Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping		
7 1	2	321500000	A		
14 2	2	282500000	A	B	
14 1	2	221500000	A	B	
0 2	2	218000000		B	
0 1	2	196500000		B	C
7 2	2	186000000		B	C
21 1	2	100000000			C D
21 2	2	97000000			C D
28 2	2	83000000			D
28 1	2	4650000			D

Means that do not share a letter are significantly different.

APPENDIX H

EFFECT OF STORAGE AND YOGURT COMPOSITION ON SURVIVAL OF TOTAL BACTERIA IN YOGURT SAMPLES

Table H. 1 ANOVA Table for the effect of storage and yogurt composition on survival of total bacteria in yogurt samples

General Linear Model: Total Bacteria versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	2,89415E+18	7,23537E+17	5,38	0,004
Yogurt	3	9,68652E+17	3,22884E+17	2,40	0,098
Day*Yogurt	12	2,99445E+18	2,49537E+17	1,86	0,107
Error	20	2,69015E+18	1,34508E+17		
Total	39	9,54740E+18			

Table H. 1 ANOVA Table for the effect of storage and yogurt composition on survival of total bacteria in yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	256672500	57988717	4,43	0,000	
Day					
0	530952500	115977434	4,58	0,000	1,60
7	-101772500	115977434	-0,88	0,391	1,60
14	-90722500	115977434	-0,78	0,443	1,60
21	-124547500	115977434	-1,07	0,296	1,60
Yogurt					
1	-133619500	100439404	-1,33	0,198	1,50
2	-155500500	100439404	-1,55	0,137	1,50
3	225292500	100439404	2,24	0,036	1,50
Day*Yogurt					
0 1	-262005500	200878809	-1,30	0,207	2,40
0 2	-326124500	200878809	-1,62	0,120	2,40
0 3	937082500	200878809	4,66	0,000	2,40
7 1	43069500	200878809	0,21	0,832	2,40
7 2	55850500	200878809	0,28	0,784	2,40
7 3	-165192500	200878809	-0,82	0,421	2,40
14 1	27619500	200878809	0,14	0,892	2,40
14 2	43400500	200878809	0,22	0,831	2,40
14 3	-176242500	200878809	-0,88	0,391	2,40
21 1	99494500	200878809	0,50	0,626	2,40
21 2	109375500	200878809	0,54	0,592	2,40
21 3	-327917500	200878809	-1,63	0,118	2,40

Table H. 1 ANOVA Table for the effect of storage and yogurt composition on survival of total bacteria in yogurt samples (Continued)

Regression Equation

Total Bacteria = 256672500 + 530952500 Day_0 - 101772500 Day_7
- 90722500 Day_14
- 124547500 Day_21 - 213910000 Day_28 - 133619500 Yogurt_1
- 155500500 Yogurt_2 + 225292500 Yogurt_3 + 63827500 Yogurt_4
- 262005500 Day*Yogurt_0 1 - 326124500 Day*Yogurt_0 2
+ 937082500 Day*Yogurt_0 3 - 348952500 Day*Yogurt_0 4
+ 43069500 Day*Yogurt_7 1 + 55850500 Day*Yogurt_7 2
- 165192500 Day*Yogurt_7
3 + 66272500 Day*Yogurt_7 4 + 27619500 Day*Yogurt_14 1
+ 43400500 Day*Yogurt_14 2 - 176242500 Day*Yogurt_14 3
+ 105222500 Day*Yogurt_14 4 + 99494500 Day*Yogurt_21 1
+ 109375500 Day*Yogurt_21 2 - 327917500 Day*Yogurt_21 3
+ 119047500 Day*Yogurt_21 4 + 91822000 Day*Yogurt_28 1
+ 117498000 Day*Yogurt_28 2 - 267730000 Day*Yogurt_28 3
+ 58410000 Day*Yogurt_28 4

Fits and Diagnostics for Unusual Observations

Obs	Total Bacteria	Fit	Resid	Std Resid	
3	3000000000	1950000000	1050000000	4,05	R
23	900000000	1950000000	-1050000000	-4,05	R

R Large residual

Table H. 1 ANOVA Table for the effect of storage and yogurt composition on survival of total bacteria in yogurt samples (Continued)

Comparisons for Total Bacteria

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
0	8	787625000	A
14	8	165950000	B
7	8	154900000	B
21	8	132125000	B
28	8	42762500	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
3	10	481965000	A
4	10	320500000	A
1	10	123053000	A
2	10	101172000	A

Means that do not share a letter are significantly different.

Table H. 1 ANOVA Table for the effect of storage and yogurt composition on survival of total bacteria in yogurt samples (Continued)

Tukey Pairwise Comparisons: Day*Yogu rt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping	
0 3	2	1950000000	A	
0 4	2	502500000	A	B
0 1	2	392000000		B
14 4	2	335000000		B
21 4	2	315000000		B
0 2	2	306000000		B
7 4	2	285000000		B
7 3	2	215000000		B
14 3	2	215000000		B
28 4	2	165000000		B
21 1	2	98000000		B
21 2	2	86000000		B
7 1	2	64350000		B
14 1	2	59950000		B
7 2	2	55250000		B
14 2	2	53850000		B
21 3	2	29500000		B
28 2	2	4760000		B
28 1	2	965000		B
28 3	2	325000		B

Means that do not share a letter are significantly different.

APPENDIX I

EFFECT OF STORAGE AND YOGURT COMPOSITION ON SURVIVAL OF MOLD AND YEAST IN YOGURT SAMPLES

Table I. 1 ANOVA Table for the effect of storage and yogurt composition on survival of mold and yeast in yogurt samples

General Linear Model: Mold & Yeast versus Day; Yogurt

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 7; 14; 21; 28
Yogurt	Fixed	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	34678415	8669604	3,88	0,017
Yogurt	3	1237906757	412635586	184,81	0,000
Day*Yogurt	12	34692520	2891043	1,29	0,294
Error	20	44655027	2232751		
Total	39	1351932719			

Table I. 1 ANOVA Table for the effect of storage and yogurt composition on survival of mold and yeast in yogurt samples (Continued)

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3832	236	16,22	0,000	
Day					
0	-894	473	-1,89	0,073	1,60
7	-780	473	-1,65	0,115	1,60
14	-328	473	-0,69	0,496	1,60
21	362	473	0,77	0,452	1,60
Yogurt					
1	-1892	409	-4,62	0,000	1,50
2	-3827	409	-9,35	0,000	1,50
3	-3819	409	-9,33	0,000	1,50
Day*Yogurt					
0 1	-1046	818	-1,28	0,216	2,40
0 2	889	818	1,09	0,290	2,40
0 3	882	818	1,08	0,294	2,40
7 1	-960	818	-1,17	0,254	2,40
7 2	777	818	0,95	0,354	2,40
7 3	774	818	0,95	0,356	2,40
14 1	-112	818	-0,14	0,893	2,40
14 2	327	818	0,40	0,694	2,40
14 3	327	818	0,40	0,694	2,40
21 1	448	818	0,55	0,590	2,40
21 2	-359	818	-0,44	0,666	2,40
21 3	-357	818	-0,44	0,667	2,40

Table I. 1 ANOVA Table for the effect of storage and yogurt composition on survival of mold and yeast in yogurt samples (Continued)

Regression Equation

Mold & Yeast = 3832 - 894 Day_0 - 780 Day_7 - 328 Day_14 + 362 Day_21
+ 1640 Day_28
- 1892 Yogurt_1 - 3827 Yogurt_2 - 3819 Yogurt_3 + 9538 Yogurt_4
- 1046 Day*Yogurt_0 1 + 889 Day*Yogurt_0 2 + 882 Day*Yogurt_0 3
- 726 Day*Yogurt_0 4 - 960 Day*Yogurt_7 1 + 777 Day*Yogurt_7 2
+ 774 Day*Yogurt_7 3 - 590 Day*Yogurt_7 4 - 112 Day*Yogurt_14 1
+ 327 Day*Yogurt_14 2 + 327 Day*Yogurt_14 3 - 542 Day*Yogurt_14 4
+ 448 Day*Yogurt_21 1 - 359 Day*Yogurt_21 2 - 357 Day*Yogurt_21 3
+ 268 Day*Yogurt_21 4 + 1670 Day*Yogurt_28 1
- 1635 Day*Yogurt_28 2
- 1626 Day*Yogurt_28 3 + 1590 Day*Yogurt_28 4

Fits and Diagnostics for Unusual Observations

Obs	Mold & Yeast	Fit	Resid	Std Resid	
4	16000	11750	4250	4,02	R
24	7500	11750	-4250	-4,02	R

R Large residual

Table I. 1 ANOVA Table for the effect of storage and yogurt composition on survival of mold and yeast in yogurt samples (Continued)

Comparisons for Mold & Yeast

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
28	8	5471,50	A		
21	8	4193,88	A	B	
14	8	3503,75	A	B	
7	8	3052,25		B	
0	8	2937,50		B	

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping		
4	10	13370,0	A		
1	10	1940,0		B	
3	10	12,3			C
2	10	4,8			C

Means that do not share a letter are significantly different.

Table I. 1 ANOVA Table for the effect of storage and yogurt composition on survival of mold and yeast in yogurt samples (Continued)

Tukey Pairwise Comparisons: Day*Yogurt

Grouping Information Using the Tukey Method and 95% Confidence

Day*Yogurt	N	Mean	Grouping
28 4	2	16600,0	A
21 4	2	14000,0	A
14 4	2	12500,0	A
7 4	2	12000,0	A
0 4	2	11750,0	A
28 1	2	5250,0	B
21 1	2	2750,0	B
14 1	2	1500,0	B
7 1	2	200,0	B
28 3	2	26,0	B
21 3	2	17,5	B
14 3	2	11,5	B
28 2	2	10,0	B
21 2	2	8,0	B
7 3	2	6,5	B
14 2	2	3,5	B
7 2	2	2,5	B
0 3	2	0,0	B
0 1	2	-0,0	B
0 2	2	-0,0	B

Means that do not share a letter are significantly different.

APPENDIX J

QUESTIONNAIRE FOR SENSORY ANALYSIS

Table J. 1 Questionnaire for sensory analysis

SENSORY ANALYSIS OF 4 DIFFERENT YOGURT SAMPLES **PANELIST NAME:**				
	1	**2**	**3**	**4**
Flavor				
Odor				
Appearance				
Texture				
General acceptability				
Notes:				
Please evaluate the yogurt samples according to : 5- Very good 4- Good 3- Normal 2- Bad 1- Very bad				

APPENDIX K

EFFECT OF YOGURT COMPOSITION ON SENSORY ANALYSİS

Table.K.1 Effect of Yogurt Composition on Sensory Analysis (Taste)

One-way ANOVA: Flavor versus Yogurt

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Yogurt	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Yogurt	3	0,3929	0,1310	0,15	0,925
Error	24	20,2857	0,8452		
Total	27	20,6786			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,919368	1,90%	0,00%	0,00%

Table.K.1 Effect of Yogurt Composition on Sensory Analysis (Taste) (Continued)

Means

Yogurt	N	Mean	StDev	95% CI
1	7	4,143	1,069	(3,426; 4,860)
2	7	4,000	1,000	(3,283; 4,717)
3	7	4,286	0,756	(3,569; 5,003)
4	7	4,000	0,816	(3,283; 4,717)

Pooled StDev = 0,919368

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
3	7	4,286	A
1	7	4,143	A
4	7	4,000	A
2	7	4,000	A

Means that do not share a letter are significantly different.

Table.K.2 Effect of Yogurt Composition on Sensory Analysis (Odor)

One-way ANOVA: Odor versus Yogurt

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0,05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Yogurt	4	1; 2; 3; 4

Table.K.2 Effect of Yogurt Composition on Sensory Analysis (Odor) (Continued)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Yogurt	3	1,429	0,4762	0,48	0,702
Error	24	24,000	1,0000		
Total	27	25,429			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1	5,62%	0,00%	0,00%

Means

Yogurt	N	Mean	StDev	95% CI
1	7	4,000	0,577	(3,220; 4,780)
2	7	3,571	1,134	(2,791; 4,352)
3	7	3,714	0,951	(2,934; 4,494)
4	7	4,143	1,215	(3,363; 4,923)

Pooled StDev = 1

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
4	7	4,143	A
1	7	4,000	A
3	7	3,714	A
2	7	3,571	A

Means that do not share a letter are significantly different.

Table.K.3 Effect of Yogurt Composition on Sensory Analysis (Appearance)

One-way ANOVA: Apperance versus Yogurt

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Yogurt	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Yogurt	3	0,7143	0,2381	0,91	0,451
Error	24	6,2857	0,2619		
Total	27	7,0000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,511766	10,20%	0,00%	0,00%

Means

Yogurt	N	Mean	StDev	95% CI
1	7	4,429	0,535	(4,029; 4,828)
2	7	4,286	0,488	(3,886; 4,685)
3	7	4,571	0,535	(4,172; 4,971)
4	7	4,714	0,488	(4,315; 5,114)

Pooled StDev = 0,511766

Table.K.3 Effect of Yogurt Composition on Sensory Analysis (Appearance) (Continued)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
4	7	4,714	A
3	7	4,571	A
1	7	4,429	A
2	7	4,286	A

Means that do not share a letter are significantly different.

Table.K.4 Effect of Yogurt Composition on Sensory Analysis (Texture)

One-way ANOVA: Texture versus Yogurt

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Yogurt	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Yogurt	3	1,250	0,4167	0,47	0,708
Error	24	21,429	0,8929		
Total	27	22,679			

Table.K.4 Effect of Yogurt Composition on Sensory Analysis (Texture) (Continued)

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,944911	5,51%	0,00%	0,00%

Means

Yogurt	N	Mean	StDev	95% CI
1	7	4,429	0,787	(3,691; 5,166)
2	7	3,857	0,900	(3,120; 4,594)
3	7	4,000	1,155	(3,263; 4,737)
4	7	4,143	0,900	(3,406; 4,880)

Pooled StDev = 0,944911

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
1	7	4,429	A
4	7	4,143	A
3	7	4,000	A
2	7	3,857	A

Means that do not share a letter are significantly different.

Table.K.5 Effect of Yogurt Composition on Sensory Analysis (General acceptability)

One-way ANOVA: General Acceptability versus Yogurt

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0,05

Equal variances were assumed for the analysis.

Table.K.5 Effect of Yogurt Composition on Sensory Analysis (General acceptability) (Continued)

Factor Information

Factor	Levels	Values
Yogurt	4	1; 2; 3; 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Yogurt	3	0,9643	0,3214	0,84	0,483
Error	24	9,1429	0,3810		
Total	27	10,1071			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,617213	9,54%	0,00%	0,00%

Means

Yogurt	N	Mean	StDev	95% CI
1	7	4,429	0,535	(3,947; 4,910)
2	7	4,000	0,816	(3,519; 4,481)
3	7	4,000	0,577	(3,519; 4,481)
4	7	4,286	0,488	(3,804; 4,767)

Pooled StDev = 0,617213

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Yogurt	N	Mean	Grouping
1	7	4,429	A
4	7	4,286	A
3	7	4,000	A
2	7	4,000	A

Means that do not share a letter are significantly different.