DEVELOPING SPREADABLE PEANUT BUTTERS INCORPORATED WITH THE ENCAPSULATED POTENTIAL PSYCHOBIOTIC *Lactococcus lactis* C19. 1 STRAIN

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ABSTRACT

DEVELOPING SPREADABLE PEANUT BUTTERS INCORPORATED WITH THE ENCAPSULATED POTENTIAL PSYCHOBIOTIC *Lactococcus lactis* C19.1 STRAIN

In recent years, the partially processed food market has increased, partly due to the demand for fresh-cut or dried products. However, there is an increasing demand for foods enriched with physiologically active ingredients such as probiotics and prebiotics. From the nutrional point view, these two functional factors, have created a new market area for fruit and snack products. Peanuts have high nutritional value (24% protein, 45% fat and 13% fiber) as well as high amounts of thiamine and niacin). It is also known to reduce the risk of prostate, liver, colon and lung cancer and stress-related diseases. In this study, as a model bacteria *Lactococcus lactis* C19.1 strain isolated from Turkish cottage cheese has been impregnated with peanut and cashew nut butter after encapsulation. In the selection of the *Lactococcus lactis* strain, porbiotic and anti-stress properties were considered. To increase the viability of the strain, the encapsulation was carried by whey protein-xylan complex as the wall material. After encapsulation, the probiotic strain was added to the peanut and cashew nut butter formulation. In addition, in order to determine the encapsulation efficiency, it was impregnated to the butters the free cell form. The results revealed that encapsulation increased the shelf life stability of the psychobiotic cell. The shelf life of the peanut butters containing free cell of *L. lactis* was determined as 30 days that of 45 days in encapsulated cell containing samples. In addition, *in vitro* analyzes revealed that encapsulation protected probiotic cells in simulated gastrointestinal system and, the viability was between 10^6 – 10^7 CFU/g. However, the viability rate was found to be 10^5 in fort he free cell containing samples after digestion test. Cashews and peanut butters containing encapsulated cell came to the fore, as 10^5 CFU/g remained below the 10^6 CFU/g, which is the condition of being probiotic.

ÖZET

ENKAPSÜLLENMİŞ POTANSİYEL PSİKOBİYOTİK *Lactococcus lactis* C19.1 SUŞU İLAVELİ SÜRÜLEBİLİR FISTIK EZMELERİNİN GELİŞTİRİLMESİ

Son yıllarda, kısmen işlenmiş gıda piyasası, kısmen taze kesilmiş veya kurutulmuş ürün talebine bağlı olarak artış göstermiştir. Bununla birlikte, probiyotik ve prebiyotikler gibi fizyolojik olarak fonksiyonel bileşenlerce zenginleştirilmiş gıdalara yönelik artan bir talep bulunmaktadır. Besinsel açıdan bakıldığında bu iki fonksiyonel faktör, bu tür bileşenlerle zenginleştirilmiş meyve ve çerez ürünleri için yeni bir pazar alanı oluşturmuştur. Fıstık yüksek besinsel değere (%24 protein, %45 yağ ve %13 lif) aynı zamanda yüksek miktarda tiamin ve niasin) sahip bir kuruyemiştir. Ayrıca prostat, karaciğer, kolon ve akciğer kanseri ve strese bağlı hastalık riskini azalttığı da bilinmektedir. Bu çalışmada, probiyotiklerin sağlık üzerindeki etkileri göz önüne alınarak fıstık ve kaju ezme formülasyonunda Türk çökelek peynirinden izole edilen model bakteri *Lactococcus lactis* C19.1'in serbest ve enkapsüle halde ilavesi gerçekleştirilmiştir. Probiyotik ve antistres özelikleri üzerinde yapılan çalışmaların yoğun olduğu *Lactococcus lactis* suşunun seçilerek ve stabilitesinin artırılması amacıyla peyniraltı suyu protein-ksilan kompleksi ile enkapsüle edilip fıstık ve kaju ezmelerine ilave edilmiştir. Ayrıca enkapsülasyon verimliliğinin belirlenmesi amacıyla serbest hücrelerde ezme formülasyonuna katkılanmıştır. Elde edilen bulgulara göre enkapsülasyon işleminin ezmelerin probiyotik raf ömrünü arttırdığı belirlenmiştir. Serbest hücre psikobiyotik eklenmiş fıstık ezmelerinin raf ömrü 30 günde kalırken, enkapsüle edilmiş psikobiyotik eklenen fıstık ezmelerinin raf ömrü 45 gün olmuştur. Ayrıca *in vitro* analiz sonuçlarına göre enkapsülasyon işleminin simüle sindirim ortamında probiyotik hücrelerin dayanımını artırdığı görülmüştür. Sindirim testi sonucunda enkapsüle probiyotik içeren fıstık ve kaju ezmelerinin canlılık seviyesi 10^6 – 10^7 KOB/g arasındatespit edilirken bu sayı serbest hücre içeren ezme örneklerinde 10^5 KOB/g seviyesine düşmüştür. 10^5 KOB/g, probiyotik olma koşulu olan 10^6 KOB/g'ın altında kaldığı için enkapsüle kaju ve fıstık ezmeleri öne çıkmıştır.

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CHAPTER 1

INTRODUCTION

In recent years, functional foods, which we can be defined as healthy foods, have gained great importance in consumption and international trade. Increasing education rate, technological developments and improvements in the field of health, and changes in life expectancy have increased the trends towards weight control and health awareness. This increase has brought with it the careful consumption of food, which has made the production and consumption of functional food becoming as the number one area of the food industry (Dillard and German, 2000). In the mid-1980s, the term functional food was discovered for the first time in Japan, and this term was defined as industrial food containing nutritious food components that help the development of body functions (Swinbanks and O' Brien, 1993). Functional foods contain dietary fibers, vitamins, minerals and many probiotic and prebiotic supplements. They are generally found in fermented products such as milk, yogurt, kefir, and supplementary food products such as sports drinks and baby foods. As a result of the increasing interest in prebiotic and probiotic products, studies in this field have also on the march. Researchers have succeeded in isolating new generation probiotic strains. As a result, the market for products containing probiotics has grown considerably.

Probiotics are live microorganisms found naturally in food products or added to them (Parka, et al., 2018). The international trend or scientific consensus states that probiotic foods must contain 10^6–10^7 CFU/g in order to be classified as healthy products and to benefit the human body (Champagne et al., 2011). According to the Turkish Food Codex, it is stated that for a food to be called a probiotic food, it must contain at least $1.0x10^6$ CFU/g of live probiotic microorganisms. According to many studies, probiotics improve the environment of the human gut microbiota and help to stay healthy by protecting the organisms in the gut microbiota (Hemarajata & Versalovic, 2013). *Lactobacillus, Leuconostoc* and *Bifidobacteria* grow well in the human intestine and thus prevent the development of pathogens harmful to the intestine. These microorganisms prevent intestinal tract infections, improve lactose metabolism (Jiang, Li, Zhang, & Ren,

2008), lower serum cholesterol levels, increase immunity, stimulate calcium absorption, increase protein digestibility and vitamins (B vitamins, nicotinic acid and folic acid) counteracts the effects of foodborne pathogens and can be used for these purposes alone or in combination with other probiotics. Health and medical professionals are increasingly focusing on the effects of probiotics on human health in their studies (Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015). Evidence has also been published showing possible antidepressant and anti-stress effects of probiotic supplements. In two recently published research articles, a meta-analysis examining the effect of probiotics on depressive symptoms (e.g. behavior, mood) with randomized controlled trials has been conducted, and studies have proven beneficial effects of probiotics on depressive responses (Huang et al. et al. 2016, Rios. et al., 2017, Wallace and Milev, 2017).

Encapsulation is a widely used method in the food industry to be a protector for liquid and solid bioactive components from harmful environmental (oxygen, free radicals and light, etc.) factors. (Desai & Park, 2005). The efficiency of encapsulation depends on many factors such as encapsulation method, encapsulation material, capsule membrane material and functionality (John, Tyagi, Brar, Surampalli, & Prévost, 2011). Depending on the size of the capsules produced, encapsulation can be broadly classified into three types: macroencapsulation (millimeter to centimeter) and microencapsulation (1–1000 μm) and nanoencapsulation (<1 μm) (Heidebach, Först, & Kulozik, 2012).

There are various encapsulation methods and types used to protect probiotics against environmental factors. Spray drying, coacervation, emulsification, extrusion and lyophilization methods are the main encapsulation methods used for probiotics. Since populations of probiotic microorganisms can be rapidly affected by the encapsulation processes, it is of great importance to choose a method suitable for the characteristics of the probiotic used. Emulsification is a suitable method for probiotic bacteria in situations where protection is needed such as processing and storage of food, passage through the human gastrointestinal tract. Oil-in-water (W/O/W) emulsions are heterogeneous liquid dispersions of oil globules dispersed in an aqueous phase. According to Zanetti et al. (2001), in order to carry out the emulsification method, the core is first dissolved in an organic solvent with the membrane material. This dispersion solution is then emulsified in the water and oil phases containing the emulsion stabilizer. The liquid part of the

organic solvent formed was evaporated and the remainder forms the encapsulated core globules. This technique is often preferred for simplicity of method procedures and easy availability of ingredients in formulation preparation. Emulsification technique is mainly used to encapsulate microorganisms, enzymes, vitamins and minerals (Azerado et al., 2005). Song et al. (2013) found that probiotics microencapsulated with emulsion liquid containing alginate-chitosan mixture were more resistant under stimulated gastrointestinal tract conditions.

The development of a functional foodstuff with high viable microorganism content from peanut butter is extremely interesting, not only because it represents an opportunity in the functional food industry, but also it can be used to reduce the disadvantages and side effects of traditionally used treatments. As technology advances and access to information becomes easier, people have started to become more conscious. Consumers have tended to research the ingredients of the foods they eat and to avoid the side effects of drugs by using less drugs. This, in turn, has increased the demand for functional foods that are rich in ingredients and that may be beneficial in the treatment of some diseases. Peanut butter is a food that has been in our lives for a long time and has become an indispensable part of breakfast. Peanut butters can be made with different kinds of snacks such as peanuts, cashews, hazelnuts etc.

The production of snacks with the impregnation of probiotic and psychobiotic *Lactococcus lactis* strains in peanut and cashew nut butter is a novel since there have not been similar studies present in the literature. Therefore, this project is based on exploring the anti-stress property of *Lactococcus lactis*. The aim of the this thesis is to develop a functional snack/breakfast spread product that can be supplied easily either from a school canteenor shopping malls by every individual with stress problems. In this study, *Lactococcus lactis* was encapsulated within a protein complex containing xylan and added into cashew nut and peanut butter formulation. The stability of probiotics in terms of viability was followed during the storage period and *in vitro* gastrointestinal system.

CHAPTER 2

PROBIOTICS AND PSYCHOBIOTICS

2.1 Definition and Characteristics of Probiotics

In 2500 BC, one of the oldest medical sciences, the process of fermentation of milk, was made. It is a known fact that yogurt is beneficial for our health. But it was not known why it was healthy. What is the ingredient that makes yogurt healthy? In the studies conducted in the early period of the last century, it has been proven that, *Bifidobacteria*, LAB group bacteria members are found as the predominant component in the intestinal microflora of infants in the isolations made from the intestinal layer of breastfed infants (Fooks and Gibson, 2002; O'Sullivan et al., 1992; Harmsen-Hermie et al., 2000; Ishiba-shi and Shimamura, 1993).

The human gut hosts one of the most complex and large ecosystems, consisting of 10^{13}–10^{14} microorganisms, 10 times more than the number of eukaryotic cells in the human body (Leung & Thuret, 2015; Qin et al., 2010). An adult human gut microbiota contains approximately 500-1000 bacterial species (Leung & Thuret, 2015; Palmer et al., 2007; Jasarevic et al., 2015). These species are presented in Table 2.1. These microorganisms, which are also found in a wide variety of food products, pass through our intestines and descending our intestinal microflora every day, where they interact and pass into our blood, providing many benefits to our body (Hill et al., 2014). Many of these microorganisms are known as probiotics. Probiotics are defined as "live microorganisms that, when ingested in sufficient quantities, provide health benefits to the host organism" (Hill et al., 2014).

In the human intestine, probiotic bacteria such as *Lactobacillus, Leuconostoc* and *Bifidobacteria* develop well and prevent the development of intestinal pathogens. These microorganisms can be used alone or in combination with probiotics for some functions such as increase immunity, lower cholesterol levels, prevent intestinal tract infections, increase protein digestibility, contribute lactose metabolism positively (Jiang, Li, Zhang, & Ren, 2008), activate calcium absorption, vitamin B, nicotinic acid and folic acid),

eliminating the harmful effects of foodborne pathogens. In the ongoing studies, the use of probiotics instead of antibiotics has come to the fore (Isolauri, 2001; Gibson & Rastall, 2004). However, there are evidence that proposes possible antidepressant effects caused by probiotic supplement. For example, based on the last two research publications, randomized controlled studies, which examine the effect of probiotics on symptoms of depression, suggest that a meta-analysis (e.g. mood, comprehension), probiotics, clinical and nonclinical participants may have beneficial effects on depressive symptoms (Wallace & Milev, 2017; Rios et al., 2017; Huang et al. 2016).

Table 2.1 Lactic acid bacteria species used in probiotic foods (Parvez et al., 2006).

Lactobacillus sp.	***Bifidobacterium sp.***	***Enterococcus sp.***	***Streptococcus sp.***
L. acidophilus	*B. bifidum*	*Ent. faecalis*	*S. cremoris*
L. casei	*B. adolescentis*	*Ent. faecium*	*S. salivarius*
L. delbrueckii ssp. (bulgaricus)	*B. animals*		*S. diacetylactis*
L. cellobiosus	*B. infantis*		*S. intermedius*
L. curvatus	*B. thermophilum*		
L. fermentum	*B. longum*		
L. lactis			
L. plantarum			
L. reuteri			
L. brevis			

Few studies have examined the psychobiotic effects of prebiotics. These studies have shown that galactooligosaccharides (GOS) and fructooligosaccharides (FOS), which are a food source for *Bifidobacteria* and *Lactobacilli* and stimulate their activity and spread in the intestine, have psychobiotic properties (Bermúdez-Humarán et al., 2019).

2.2 Psychobiotics

Depression (major depressive disorder, MDD) is a serious medical illness that negatively affects thoughts, behaviors, emotions, motivation, and sense of well-being (De Zwart et al., 2019). Today, depression is accepted as a civilization disease due to its prevalence and incidence, especially in developed countries. Globally, approximately 300 million people, or 4.4% of the world's population, suffer from depression (Global Burden of Disease Study 2015). Many *in vivo* and clinical studies have demonstrated the important role of stress in the onset of depression (Nemeroff et al., 2005; Herbet et al., 2017).

Psychobiotics are an important subclass of probiotics that have beneficial effects on individuals' mental health. Dinan et al. (2012) defined psychobiotics as a class of probiotics that have positive effects on mental health when taken into the body in sufficient quantities. These effects are presented in Table 2.2. They differ from other classes of probiotics in their superior ability to produce or stimulate the production of neurotransmitters, ɣ-aminobutyric acid (GABA), short-chain fatty acids, enteroendocrine hormones, and anti-inflammatory cytokines. Because of these potential abilities, psychobiotics have a wide range of applications, from alleviating mood and stress, to being supportive and complementary in the therapeutic treatment of various neurodevelopmental and neurodegenerative disorders. The regulatory effect of psychobiotics is not limited to the neuroimmune axes such as the hypothalamic-pituitary-adrenal axis, the sympathoadrenal medullary axis and the inflammatory reflex, they have positive effects on diseases including learning, nervous system, memory and cognitive. Psychobiotics thus provided a comprehensive perspective on changing the existing symbiosis relationship between bacteria and humans. From this new perspective, this relationship appears to be a type of relationship in which humans are affected but bacteria are not, rather than a pure symbiosis. The most known psychobiotic bacteria families are *Lactobacilli, Escherichia, Streptococci, Bifidobacteria, and Enterococci* (Sharma et al., 2021).

Table 2.2 Effect of Psychobiotics on Stress

Psychobiotic	Disease	Clinical Study	Test Group	Conclusion	Reference
Lb. rhamnosus R0011 and Lb. helveticus R0052	GI diseases depending on chronic stress		In rats	They increased mucosal defences and protected the intestines from this stress.	(Gareau et al. 2011)
Lb. rhamnosus (R0011) + *Lb. helveticus* (R0052)	Memory disorders because of exposed to stress	Behaviour Test	In mice having Citrobacter rodentium infection	They protected the disorder	(Mckernan et al. 2010)
Lb. helveticus NS8	on body weight, stress, anxiety, anti-inflammatory cytokine IL-10.	Behaviour Test	In older rats exposing to stress	Antidepressant effect was showed. It also regulates 5-HT and its synthesis.	(Liang et al. 2015)
B. longum 1714 and *B. breve* 1205	Psychological disorders related to stress were investigated.		In mice	*B. longum* 1714 was showed positive and therapeutic effects in diseases.	(Savignac et al. 2015)
L. helveticus ROO52 and *B. Longum* R0175		Randomize double-blind Hopkins Symptom Checklist, Hospital Depression and Anxiety Scale, Perceived stress scale, Coping Checklist and Urinary free cortisol for human, anxiety testing for rats	In rats and human	Taken in combination can be prevent anxiety disorder in rat and can be beneficial effect as psychological for human	(Messaoudi et al., 2011).
Lb. acidophilus Rosell-52 and *B. longum* Rosell-175	Diseases such as abdominal pain and nausea caused by stress	Randomized, double-blinded study	Healthy individuals	These disorders were treated	(Diop et al. 2008)

In an experiment conducted with the participation of 140 medical school 5th grade students in Japan, it was determined that the students were very stressed before their full course exam day, and to prevent this, they were given milk with the addition of *Lactobacillus casei* (Takada et al., 2016). The group of students who received this milk was named LcS. During eight weeks, the physical symptoms of the students when they

were stressed were measured. The control group, called the placebo group, weren't given milk. At the end of eight weeks, the stress symptom values of the LcS group were significantly lower than the stress symptom values of the placebo group (Takada et al., 2016).

In a study, *Lb. helveticus* NRLL B-4526*, Lb. rhamnosus* NRRL B-442*, Lb. delbrueckii subsp. bulgaricus* NRRL B-548*, and Lc. lactis subsp. lactis* CECT 4432 strains were used to compare the GABA concentrations produced by these strains at 96 weeks (Özer, 2019). It was desired to determine the GABA producing abilities of these four strains. After 96 weeks of studies, the GABA producing abilities of these four strains have been proven. Also *Lb. rhamnosus* NRRL B-442 was the strain that have the highest GABA production concentration with 52,950 mg/L (Özer, 2019).

The effect of probiotic-added fermented milk product consumed by healthy female individuals for 4 weeks on brain function, emotional changes and attention of individuals was investigated (Tillisch et al., 2013). In this study, *Bifidobacterium animalis lactis, Streptococcus thermophiles, Lactobacillus bulgaricus* and *Lactococcus lactis* were used as probiotics. There were 36 participants, 12 women who consumed probiotic-containing fermented milk products twice a day for four weeks, 11 women who consumed regular fermented milk runes, and 13 women who did not consume anything. At the end of four weeks, increased activity was observed in the brain functions of individuals who consumed fermented milk products containing probiotics (Tillisch et al., 2013).

2.2.1 Mode of Action of Psychobiotics

There is bidirectional signal communication between the metabolically complex gut microbiota, the gut and the brain. This signal communication is provided by the combination of immunological, neural and hormonal signals between this triple axis (microbiota-gut-brain axis). The gut microbiota and its metabolites influence GI activities such as brain motility, gut permeability, blood flow, microbiota interactions and immunomodulation, and also support brain function and development by modulating the central nervous system.

Findings from many recent studies support the prediction that microorganisms communicating via the gut-brain axis can alter mental and emotional processes (Collins et al., 2012). Although it is well known that gut pathogens have adverse effects on mental function, new evidence is emerging that the gut microbiota similarly has beneficial effects on mental health.

Intestinal bacteria also produce a number of neurotransmitters through the metabolism of indigestible fibers. Intestinal bacteria metabolize indigestible fibers and produce transmitters of GABA, dopamine, noradrenaline, serotonin, and acetylcholine (Trzeciak & Herbet, 2021). For example, the *Bifidobacteria* produces GABA, the *Escherichia* produces noradrenaline and serotonin, the members of the *Bacillus* produce dopamine and noradrenaline, the *Enterococcus* and *Streptococcus* produce serotonin, and the *Lactobacilli* produces GABA and acetylcholine (Trezeciak & Herbet, 2021). There is no direct evidence yet that these neurotransmitters modulate synaptic activity in proximal neurons of the enteric nervous system, but it is likely to be proven in future research.

Generally, psychobiotic research is conducted on rodent models. Rodent behavior tests and rodent stress induction are used to evaluate the depression, anxiety and motivation of rodents (Sarkar et al., 2016). The psychophysiological effects of psychobiotics fall into three categories: (i) Psychological effects on mental and emotional states. (ii) Inflammations manifested by abnormal cytokine concentration and systemic effects on the hypothalamic-pituitary-adrenal axis and glucocorticoid stress response Proinflammatory cytokines exert a strong and positive effect in psychiatric conditions (such as depression). For example, injection of interferon-α, a proinflammatory cytokine, induced depression by acting as an antidepressant. (iii) Neural and mental effects of psychobiotics on neurotransmitters and proteins (Sarkar, Lehto et al., 2016). These neurotransmitters include the protein γ-aminobutyric acid (GABA) and its glutamate. They control the balance of neural excitation and inhibition. Because they are proteins, these proteins contain brain-derived neurotrophic factor, which has a key role in memory and learning processes (Sarkar et al., 2016). This factor decreases in the presence of depression and anxiety, but this is a reversible decrease.

Action mechanism of potential psychobiotics effect was presented in Figure 2.1. This action decreases proinflammatory cytokines and concentrations of glucocorticoids.

They increase anti-inflammatory cytokines concentrations. Anti- inflammatory cytokines provide integrity of the blood- brain barrier, gut barrier, and reduce overall inflammation (Chudzik, Orzylowska, Rola, & Stanisz, 2021). This, in turn, reduces the stress level by acting on the hypothalamus and pituitary gland. Not only probiotics but also prebiotics increase production of short-chain fatty acids. Psychobiotics reproduces neurotransmitter in the gut, including 5-HT (Serotonin), Dopamine, GABA and Noradrenaline. They modulate neurotransmission in the synapses of the enteric nervous system (Chudzik, Orzylowska, Rola, & Stanisz, 2021).

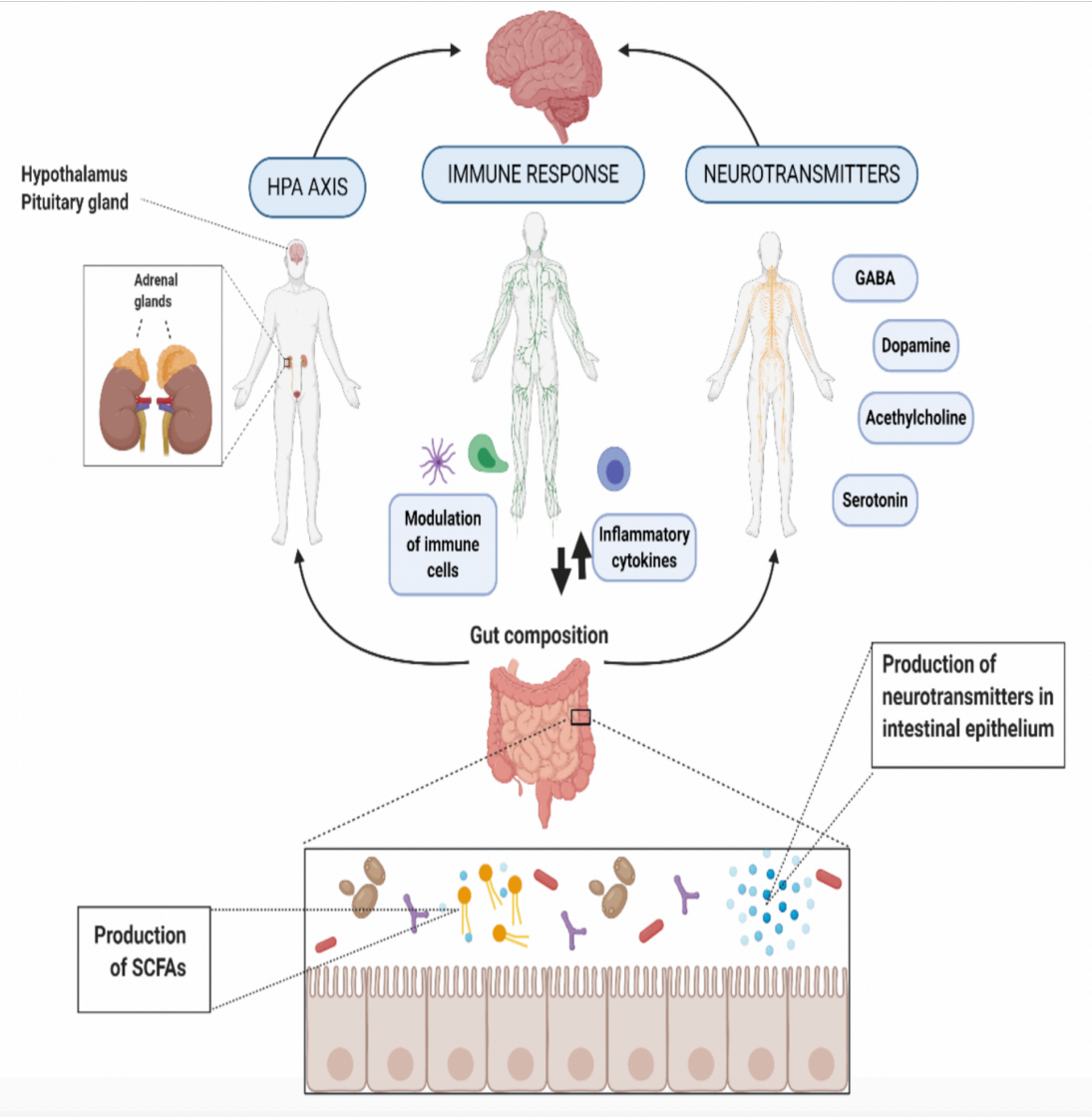

Figure 2.1 Action mechanisms by which the gut microbiota exert the potential psychobiotic effect (Toro-Barbosa et al., 2020).

2.2.2 Neurohormones and Neurotransmitters

Our gut microbiota produce certain neurochemicals that directly affect our brain functions. These bacteria are called as psychobiotics. Psychobiotics accomplish this by producing the neurohormones and neurotransmitters serotonin, dopamine and epinephrine, gamma-aminobutyric acid (GABA), acetylcholine (Table 2.3) (Del Toro-Barbarosa et al., 2020).

Table 2.3 Neurotransmitter regulatory functions and produced by probiotics (Yong et al., 2020)

NEUROTRANSMITTER	REGULATORY FUNCTIONS	PROBIOTICS	REFERENCES
Serotonin	•Impulsivity •Aggression •Appetite •Circadian rhythm •Learning •HPA axis regulation. •Mood	*L.plantarum* *L. helveticus*	Özogul (2011), Özogul et al. (2012), Oleskin et al. (2014), Carhart-Harris and Nutt (2017)
Dopamine	•Motivation •Concentration •Psychomotor speed •Ability to experience pleasure •Mood	*L. plantarum* *L. helveticus* *L. casei* *L. bulgaricus*	Dunlop and Nemeroff (2007), Özogul (2011), Oleskin et al. (2014)
Norepinephrine		*L. helveticus* *L. casei* *L. bulgaricus*	Leonard (2001), Montgomery and Briley (2011), Oleskin et al. (2014)
GABA	•Hippocampal neurogenesis •HPA axis regulation •Mood	*L. brevis* *L. rhamnosus* *L. reuteri* *L. Paracasei.* *L. plantarum* *L. Bulgaricus.* *L. helveticus* *L. casei* *L. plantarum* *L. helveticus*	Komatsuzaki et al. (2005), Luscher et al. (2011), Stromeck et al. (2011), Barrett et al. (2012), Liao et al. (2013), Lin (2013), Oleskin et al. (2014), Yunes et al. (2016)
Acetylcholine	•Cognition •Synaptic plasticity •Analgesia •Sleep •HPA axis regulation. •Mood	*L. plantarum*	Rowatt (1948), Girvin and Stevenson (1954), Pytka et al. (2016)

In a study on mice, the effects of *Lactobacillus casei* ATG-F1 (F1), *L. reuteri* ATG-F3 (F3) and *L. reuteri* ATG-F4 (F4) on dopamine and serotonin levels were investigated (Beck et al., 2019). To examine the effects of *Lactobacillus* on circulating neurotransmitters, serum dopamine and serotonin levels were measured from serum samples of each experimental group at the end of 4 weeks after each oral administration of *Lactobacillus*. ELISA test kit was used to determine dopamine and serotonin levels. There was a significant increase in serum dopamine and serum serotonin levels in the F4 group compared to the control group. However, there was no significant difference in the groups given F1 and F3 compared to the control group (Beck et al., 2019). These results suggest that oral microbial supplementation or disruption of the gut microbiota may affect host circulating neurotransmitter levels (Beck et al., 2019).

2.2.2.1 Serotonin

Serotonin (5-HT) is considered to be one of the most important neurotransmitters that can be secreted mostly by intestinal bacteria such as probiotic *Lactococcus* and *Bifidobacterium*. It is produced by the essential amino acid tryptophan in central nervous system (CNS) neurons. Changes in the amount of tryptophan in the enteric system have multiple effects on the signaling of the CNS and brain-gut system (Kennedy, P.J et al., 2017).

In a study to examine the antidepressant-like effect of probiotics and to investigate the effect of modulating gut microbiota on serotonin (5-HT) metabolism, 50 rabbits were randomly divided into four groups (Li, Wang, Huang, Li P, Zhang, 2019). While some groups of rabbits under chronic unpredictable mild stress (CUMS) were given probiotics (*Bifidobacterium longum, L. rhamnosus*) treatment, some were not, and they were subjected to a series of behavioral tests to determine the probiotic effect. During this process, the rabbits' serotonin levels were observed. According to the results, improvement was observed in CUMS-induced weight loss and depression-like behaviors of the rabbit groups treated with probiotics. Similarly, significant communication was observed between the rabbits' serotonin (5-HT) levels and the impaired gut microbiota (Li, Wang, Huang, Li P, Zhang, 2019).

In a study was conducted in the medical students under exam stress in Japan, the students were divided into two groups (Kato-Kataoka et al., 2016). The first group was given 100 ml of milk containing 1.0×10^9 CFU/mL *Lactobacillus casei* Shirota YIT 9029, while the placebo group was given 100 ml of milk that did not contain *L. casei*, and the tryptophan levels of the two groups were observed for eight weeks. According to the results, a significant increase was observed in the tryptophan levels of the group that drank milk containing *L. casei* compared to the placebo group ($p<0.05$) (Kato-Kataoka et al., 2016).

In a study was conducted in the USA on 42 healthy adults, the first group was treated with capsules containing 5×10^8 CFU *Lactobacillus johnsonii* N6.2, and the placebo group was treated with skimmed milk powder capsules (Marcial et al., 2017). After eight weeks of studies, there was a significant increase in tryptophan levels in the group treated with capsules containing *Lb. johnsonii* ($p<0.01$) (Marcial et al., 2017).

2.2.2.2 Dopamine and Epinephrine

The neurotransmitters dopamine, epinephrine, and norepinephrine produced from the amino acid tyrosine play key roles in motor control, memory functions, learning, and responses to stress. It also has positive effects on digestive events such as metabolism of fat and carbohydrates and on the cardiovascular system (Sarkar et al., 2013; Kobayashi, 2001). Norepinephrine and dopamine are noted as regulators of prefrontal cortex-dependent functions such as attention, decision making, and inhibitory control. It has positive effects on diseases such as hyperactivity, attention deficit and stress disorder (Xing et al., 2016). It has also been found that gut bacteria are a major source of norepinephrine (Sudo, 2019).

In a study conducted to demonstrate the positive effect of probiotics on the nervous system, newborn mice were divided into groups. Some groups were received probiotic *Lactobacillus rhamnosus* GG (LGG) and/or prebiotics polydextrose /galactooligosaccharide (PDX/GOS) while other groups were not (Kannampali et al., 2014). After delactation of mice, control diet (CD) was received to the first group, PDX/GOS to the second group, LGG to the third group, and PDX/GOS + LGG to the

fourth group. Levels of neurotransmitters and biogenic amines were measured in the frontal cortex, subcortex, brainstem, and cerebellum. According to the results obtained, the levels of serotonin, noradrenaline and dopamine were significantly changed in LGG-treated mice compared with the other groups (Kannampali et al., 2014).

In a recent study, male adult Flinders Sensitive Line mice (FSL; n = 22) were treated for 10 weeks with *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (10^9 or 10^{10} CFU per day) (Tillmann et al., 2018). The other group as the control group, Flinders Resistant Line mice (FRL, n= 8), was fed normally. Metabolites were measured in plasma, urine and different tissues in both groups. According to the results obtained, probiotics were observed to decrease plasma dopamine and norepinephrine in a dose-dependent manner in the FSL group (Tillmann et al., 2018).

2.2.2.3 Gamma-Aminobutyric Acid (GABA)

GABA is a non-protein amino acid produced by humans, animals, plants and different microorganisms. It has a different task in every living thing, depending on the organism from which it was produced. For example, it acts as a protective inhibitory neurotransmitter against stress in the central nervous systems of humans. It also has positive effects on human heart disease, gut regulation, and neurotransit communications (Sharon et al., 2014). For this reason, it is one of the main topics of biotechnological research in the food and pharmaceutical industries.

Microorganisms produce GABA by two pathways; 1) putrescine pathway 2) glutamic acid decarboxylase pathway. It is a small pathway that begins with the transport of putrescine into the cell with the antiporter encoded by the PuuP gene. It is a pathway used in GABA production by the bacterial strain *Escherichia coli* and the fungal strain *Aspergillus oryzae*. Glutamic acid decarboxylase pathway *Lactobacillus spp.* and is a pathway used by *Listeria monocytogenes*. It begins with the transport of the Glutamic/GABA antiporter encoded by the gadC gene into the cell (Yu et al., 2019). As with the Putrescine pathway, the GABA formed can be metabolized or enter the Krebs Cycle.

In a recent study, *Lb. helveticus* NRLL B-4526*, Lb. rhamnosus* NRRL B-442*, Lb. delbrueckii subsp. bulgaricus* NRRL B-548*, and Lc. lactis subsp. lactis* CECT 4432 strains were used to compare the GABA concentrations produced by these strains at 96 weeks (Özer, 2019). It was aimed to determine the GABA producing abilities of these four strains. After 96 weeks of studies, the GABA producing abilities of these four strains have been proven. Also *Lb. rhamnosus* NRRL B-442 was the strain that have the highest GABA production concentration with 52,950 mg/L (Özer, 2019).

In a previous study, eight GABA producer psychobiotic strains were added separately into adzuki bean milk. After 48 weeks of analysis, *Lb. rhamnosus* GG proved to be the strain with the highest GABA production concentration of 0.38 mg/ml (Song & Yu, 2018).

In another study, GABA activities of *Lb. brevis* 12005, *Lb. brevis* CECT 8183, *Lb. brevis* CECT 8181 and *Lb. brevis* CECT 8182 strains that isolated from sheep and goat cheeses were investigated (Diana, Tres, Quilez, Llombart, & Rafecas, 2014). GABA concentrations for these strains were found to be quite high as 0.83, 0.96, 0.94 and 0.94 mM, respectively (Diana, Tres, Quilez, Llombart, & Rafecas, 2014).

2.2.2.4 Acetylcholine

Acetylcholine acts as the primary neurotransmitter in the microbiota. Acetylcholine strengthens neuronal cycles and cortical dynamics during learning and can alter neural excitation. The enzymes involved in acetylcholine synthesis have also been found to be bacterial components (Picciotto et al., 2012). Its production was first discovered in a strain of *Lactobacillus plantarum* (Roshchina, 2010).

The fetus exposed to methamphetamine as a result of drug use in pregnant mothers has cognitive impairments in later life, but the mechanisms underlying these disorders are not understood (Siegel, Park, & Raber, 2011). The acetylcholine system plays an important role in cognitive function, and potential methamphetamine-induced acetylcholine changes may be associated with methamphetamine-induced cognitive impairment. In a study, the effects of methamphetamine exposure on acetylcholine levels

and neurons in adolescent mice at day 30 and in adult mice at day 90 were investigated. According to the results, methamphetamine exposure did not affect GABA concentration and neurons, but it was observed that it affected the acetylcholine mechanism and this effect was more in females (Siegel, Park, & Raber, 2011).

A study investigating the cardiovascular effects of probiotics was tested on spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKR) (Gómez-Guzmán et al., 2015). A one-to-one mixture of *L. coryniformis* CECT5711 (K8) and *L. gasseri* CECT5714 (LC9) and *Lactobacillus fermentum* CECT5716 (LC40) were used as probiotics (Gómez-Guzmán et al., 2015). Wistar Kyoto rat (WKR) and 30 SHR were randomly divided into four groups (n = 10): a control group WKR, a control group SHR, SHR group treated with LC40, and a group of SHR treated with K8/LC9 (3.3×10^{10} CFU per day). It was observed that cardiac and renal hypertrophies of SHR group rats treated with the K8/LC9 mixture were significantly reduced. The reason for this; both probiotics that consist of mixture were reversed the impaired aortic endothelium-dependent relaxation, which is the disease of the SHR group, to acetylcholine (Gómez-Guzmán et al., 2015).

CHAPTER 3

MICROENCAPSULATION

Microencapsulation for probiotics, also for psychobiotics, is a process in which probiotic cells are incorporated into the capsule material, which ensures the survival and resistance of the probiotic in harsh conditions such as the gastrointestinal tract. The reason is that, the gastrointestinal tract pH has depth and it's making it difficult for probiotics to survive in these harsh conditions. This is because the gastrointestinal tract has a pH too low for probiotics to withstand. It also contains physical and biochemical metabolic activities that will negatively affect the viability of probiotics. A microcapsule can be of various sizes, from nanometers to millimeters. It is a very thin, spherical shaped and durable structure that is semi-permeable or completely impermeable. It can be formed with capsule materials such as whey protein, xylan, alginate, chitosan alone or in combination. Due to the fact that whey proteins are a successful material as a carrier, the tendency for this material is quite high (Heidebach et al., 2009). There are many encapsulation methods used for the microencapsulation process, such as spray drying, freeze drying, emulsion technique. These methods are selected according to the characteristics of the probiotic used and the food to be incorporated as a carrier matrix, e.g. emulsion technique for yoghurt product. Encapsulated bioactives are used in the food industry for many preservation measures such as extending shelf life, preserving taste and odor, preventing oxidative reactions.

3.1 Microencapsulation of Probiotics

There are many types of microencapsulation methods used to protect probiotics against the harsh conditions of the gastrointestinal tract, such as emulsion technique, freeze drying, spray drying, extrusion method etc. When choosing a method, the characteristics of the bacteria used should be considered. Emulsion technique is very useful in industrial conditions such as food processing, storage, and in conditions of passage through the human gastrointestinal tract. Generally used emulsion types are water-oil type emulsions. In these emulsion techniques, oil globules are dispersed in an

aqueous solution as dispersion. Then the aqueous portion is evaporated to obtain capsules. The emulsification technique was mainly used to encapsulate microorganisms, enzymes, vitamins and minerals (Azerado, 2005). For probiotics, it is appropriate to encapsulate at low ambient temperature, mild conditions and anaerobic or low oxygen levels. This also applies after the encapsulation process, the capsules should be stored between 4°C and 25°C. Because this is of great importance for the viability of bacteria. In order to prevent contamination that may occur during the encapsulation process, the materials used and the encapsulation environment must be sterile and aseptic conditions must be applied. Adding antioxidants to the capsule material to prevent contamination can partially achieve this. The shaking process in the hardening procedure applied after the capsule is formed should be adjusted according to the size of the capsule (Badalan, Bottausci, Ghigliotti, Achard, & Balarac, 2022).

3.2 Microencapsulation Materials

When choosing the coating material in the encapsulation process, it must have good rheological properties and thus be easy to work with. The coating material should not interact with the coated bioactive during storage and should maintain its stability under storage conditions. It should have emulsion and dispersion properties and be stable in these properties. In order to facilitate bacterial viability count, it should be dissolved in the desired solvent. Also, the cost should not be expensive. Coating materials can also be used in combination, as not all of these properties can be found in a single coating material. For example, it can be modified cellulose, which stands out with its physical and mechanical properties (Desai & Park, 2005). In the microencapsulation process, carbohydrates such as pectin, pullulan, xylan and whey proteins, casein and caseinates are generally preferred as coating materials.

3.2.1 Alginate

Alginate is a polysaccharide naturally produced from algae, formed by various amounts and distributions of α -L guluronic and β-D mannuronic acids. The acids it consists of are helpful in determining its functional structure. Hydrogels of alginate are used in cell encapsulation, and calcium alginate sub-compound is used in encapsulation

of probiotic cells because of being affordability, biocompatibility and non-toxicity (Rowley et al., 1999; Krasaekoopt et al., 2003). Alginate has advantages as well as disadvantages such as alginate is not resistant to low pH conditions (Mortazavian et al., 2008). This is a confusing situation considering that the capsules formed in probiotic bacteria encapsulation will meet with the acidic environment of the stomach. In addition, the structure of alginate is a highly porous structure. Considering that the purpose of encapsulation is to cut off contact with the external environment, it is thought that the porous structure will bring problems with it (Gouin, 2004). However, some researchers think that this problem can be overcome by using alginate in combination with other capsule materials (Krasaekoopt et al., 2003). Some researchers argued that alginate-starch combination would be effective in solving this problem (Krasaekoopt et al., 2003; Truelstrup-Hansen et al., 2002; Sun & Griffiths, 2000; Sultana et al., 2000).

3.2.2 Chitosan

Chitosan is a linear polysaccharide composed of glucosamine units. Chitosan is very useful as an encapsulation material, as it provides good protection in the gastrointestinal tract and thus allows live bacterial cells to pass easily into the colon. At the same time, it has the feature of being the only water-soluble polysaccharide available in the industry due to the cations in its structure (Choi, Lee, 2020). It is the second most abundant in nature after cellulose. It is sensitive to pH due to the D-glucosamine amino group in its structure. In addition, inhibitory effects were found on lactic acid bacteria, which is a highly preferred bacterial group for encapsulation (Groboillot et al. 1993). But it is very useful for vitamin encapsulation in the food and pharmaceutical industries.

In a study, *Lactococcus lactis* was microencapsulated in cross-linked chitosan membranes by emulsification (Groboillot et al., 1993). Chitosan has been modified and optimized to provide biocompatible conditions. As a result of the studies, chitosan cross-linked with hexamethylene diisocyanate or glutaraldehyde, strong membranes with a narrow size distribution with an average diameter of 150 μm were obtained (Groboillot et al., 1993).

A study was conducted investigate the survival of *Lactobacillus reuteri* KUB-AC5 in simulated gastrointestinal tract (Rodklongtan et al., 2014). *Lactobacillus reuteri* KUB-AC5 was successfully microencapsulated with a coating mixture consisting of alginate and chitosan using the emulsion method. Initially, there were 10 log CFU of probiotic live cells; as a result of *in vitro* studies, 8 log CFU/g viable cells were detected in jejunum and ileum. For the *in vivo* study, a randomly amplified polymorphic DNA technique based on a polymerase chain reaction was used and 5-8 log CFU of viable cells were detected in the gut (Rodlongtan et al., 2014).

3.2.3 Whey Protein

Whey protein has been used as an encapsulation material since the 1990s. In the study, milk fat was encapsulated with a coating consisting of whey proteins and 90% encapsulation efficiency was obtained (Young et al., 1993).

In another study, whey protein orange oil was used as a capsulation material and it was determined that it formed a strong barrier against oxidation (Morr & Kim, 1996). However, as a disadvantage, whey protein is not resistant to high temperatures (Sliwinski et al., 2003). Therefore, if it is to be used as an encapsulation material, methods with more normal temperatures e.g. emulsion technique should be preferred. Whey proteins are used in industry to improve the texture and nutritional value of yoghurts and ice creams, and to improve sensory properties such as taste and smell of bakery products and confectionery products.

In recent study in which whey protein was used as a free and encapsulated probiotic carrier, 10.65, 10.17 and 9.84 CFU of live cells were detected in the encapsulated probiotics *Bifidobacterium lactis* Bb-12*, Lactobacillus plantarum* NRC AM10 and *Lactobacillus acidophilus* CH-2, respectively, as a result of *in vitro* analysis (Mabrouk et al., 2021). As a result of the scan images, it was observed that the probiotic cells were completely entrapped within the wall material. A small amount of mold and yeast and spores were detected but coliform was not detected. As a result, whey protein gel supplemented with probiotics was determined to be significantly efficient and increased storage time (Mabrouk et al., 2021).

3.2.4 Xylan

The utilization ability of carbohydrates may facilitate *Lactobacillus* strains competitive advantage and continued presence in the gut. In recent years, researchers have focused on the use of secondary plant materials in encapsulation. Plant materials are mainly composed of three types of biopolymers: cellulose, lignin and hemicellulose. Beyond these biopolymers, hemicellulose is the one that receives the most attention among all celluloses (Zhu et al., 2016). Hemicellulose is the second most abundant polysaccharide in nature. Agricultural and industrial wastes e.g. sugar beet pulp, corn fiber and corn husk contain 20-40% hemicellulose. For this reason, since hemicellulose is seen as a waste, its use in the food industry has been neglected. However, the wide usage areas of hemicellulose in the food industry have been considered as they have techno-functional characteristics e.g. thickening, adhesive, emulsifying and stabilizing (Yadav, Johnston, & Hicks, 2009) and film-forming (Hansen & Plackett, 2008). Due to these properties, hemicelluloses are thought to have a notable potential for using in food & bacterial encapsulation and emulsification methods (Ebringerová, 2006, McPherson et al. 2006. Due to the presence of small amounts of lignin and proteins, hemicellulose produces stable foams and oil/water type emulsions that act as hydrophobic centers and have a film-forming effect (Ebringerová, 2006). McPherson et al. (2006) performed encapsulation processes with hemicellulose that extracted from corn stalk and gum arabic and observed that hemicellulose was more productive than gum arabic.

A promising example of biomaterial for pharmaceutical use is xylan, a largely naturally occurring hemicellulose, considered the second most abundant polysaccharide after cellulose. Xylan is a raw material known for its use in biomedical products, but is convenient in film packaging and food coating processes in the food industry (Li et al., 2011). Xylan, extracted from corn fiber, has been used as a thickener, adhesive and additive in the food industry due to its sticky structure. Because it increases the stretching-breaking resistance and spoilage sensitivity of the food to which it is added. It increases their flexing and breaking resistance and their susceptibility to biodegradation (Unlu et al., 2009).

3.3 Microencapsulation Methods

3.3.1 Spray Drying

The spray drying ist he most common encapsulation technique for probiotics. In this technique a solution, containing wall material and probiotic suspension, is prepared and atomized in to the heated chamber. The used polymer matrices used should tend to form spherical microparticles during drying, such as starch (Chen & Chen, 2007; Kailasapathy, 2009; De Vos et al., 2010). The advantages of the spray drying method comes from being fast and cost-effective. On the other hand, the applied high temperatures (>100 ºC) cause mortal effect on probiotic viability.

In a study, *Bifidobacterium animalis lactis* was encapsulated by spray drying method using mixtures containing diffrent ratios of wall materials; physicochemical and morphological properties were investigated (Rodríguez-Restrepo et al., 2017). Capsule mixtures were obtained by combining whole milk powder, soy protein isolate and skimmed milk powder with gum arabic at the ratios of 75:25, 50:50, and 25:75, respectively. The increase in gum arabic ratio in capsule mixtures provided more solubility. The application of low-soluble wall materials promoted the stability of the encapsulated probiotic strain during storage. The spray drying method has played an effective role for all capsule mixtures and its suitability for use in the food industry has been proven (Rodríguez-Restrepo et al., 2017).

In a recent study, analyzes were performed to evaluate anchovy (*Engraulis encrasicolus L.)* oil emulsion characterization and to optimize the anchovy oil microencapsulation process by spray drying (Tatar & Kahyaoglu, 2015). Efficiency (MEE), peroxide value (PV), bulk density, Carr index (CI) and dry matter parameters were used to examine the effects of inlet air temperature (160 ºC –190 ºC), peristaltic pump speed (20–40%), and coating to oil ratio (2/1–4/1) oil particle size on microencapsulation. It was observed that the inlet air temperature had a significant effect on the samples MEE, PV and CI ($P<0.05$). Besides, the peristaltic pump speed and the coating-to-oil ratio only significantly affected MEE and PV ($P<0.05$). Optimum conditions for microencapsulation of anchovy oil were found as following parameters

e.g. 164 ºC inlet air temperature, 25% peristaltic pump speed and 4/1 coating/oil ratio were found (Tatar & Kahyaoglu, 2015).

3.3.2 Spray Freeze Drying

It is a method formed by the combination of freeze drying and spray drying methods. In this method, probiotic microorganisms are taken as solution and atomized with the vapor phase of cryogenic liquid (e.g. liquid nitrogen). The resulting frozen droplet dispersion is dried by freeze-drying (Wang et al., 2006; Kailasapathy, 2009; Semyonov et al., 2010; De Vos et al., 2010). This method is quite costly and takes a lot of time, but provides a larger specific surface area and high viability.

In a study, anthocyanins obtained from the bran fraction of black glutinous rice (BGR) were encapsulated with maltodextrins with 3 different dextrose equivalents (DE), DE10, DE20 and DE30, by spray drying and freeze drying method (Laokuldilok & Kanha, 2017). Three different drying temperatures, 140 ºC, 160 ºC and 180 ºC, were used for spray drying. As the temperature increased, microencapsulation efficiency increased, but the anthocyanin content and antioxidant activities decreased. In the spray drying method, a brighter color was obtained in the samples compared to freeze drying. In addition, the anthocyanin storage capacity was lower in the freeze-drying method than in the spray-drying method (19.7-100.0 %) (Laokuldilok & Kanha, 2017).

3.3.3 Extrusion Method

The extrusion method is a physical method and can take place under aerobic or anaerobic conditions. It does not damage probiotic microorganisms and allows probiotics to maintain their viability (Krasaekoopt et al., 2003). Encapsulation uses colloids (e.g. alginate, carrageenan) and no deleterious solvents. It is a simple and inexpensive method, but it requires a lot of time due to the slow formation of microcapsules. Therefore, it is not a convenient method for large production factories.

In a study to examine the effect of alginate concentration on the microencapsulation of probiotic *Lactobacillus casei* Shirota by extrusion and emulsification methods, the

capsules were subjected to a series of analyzes such as heat treatment survival, *in vitro*, capsule morphology, encapsulation efficiency (Gul & Dervisoglu, 2017). According to the results, different alginate concentrations significantly affected the capsule's survival in the simulated gastrointestinal tract, capsule efficiency and resistance to heat treatments. The capsules obtained by the extrusion method protected the probiotic cells more than the capsules obtained by the emulsification method (Gul & Dervisoglu, 2017).

3.3.4 Emulsion Technique

Emulsification is a water-oil type colloidal system in which oil is dispersed in aqueous solution. Emulsification systems can be oil-in-water or water-in-oil type (Bai et al., 2017). It is usually made by a combination of mechanical and chemical processes. Emulsion technique is very useful in industrial conditions such as food processing, storage, and in conditions of passage through the human gastrointestinal tract. The emulsification technique was mainly used to encapsulate microorganisms, enzymes, vitamins and minerals (Azerado, 2005). Hydrophobic and hydrophilic active ingredients can be encapsulated by this method. In addition, small diameter capsule globules can be obtained in this method. On the other hand, this method is not heat resistant and the variety and number of emulsifiers used in the method is limited.

In a study, encapsulation was done with AA2G (ascorbic acid-2-glucoside), an active functional material for skin care, was used as the W1 phase, and the biodegradable triblock copolymers of PEO-PLGA-PEO and PEO-PCL-PEO were used as wall material in a gradual emulsification method (Cho et al, 2007). W1 /O/ W2 emulsion stability was observed using a ferroxil test method and online turbidity analysis. According to the results obtained, it was observed that the use of copolymer in the W1 phase was more effective and it was observed that the copolymers reduced the size of the multiple emulsions and increased the emulsion stability (Cho et al., 2007).

In a recent study, peroxidases from turnip were encapsulated by the double emulsion technique (Dahdouh et al., 2021). Morphological characterization of the microcapsules by electron microscopy showed a spherical structure. During simulated gastric digestion, the capsules provided excellent protection against peroxidase release. Encapsulated

peroxidases retained 60% of their initial activity for 80 days at 25 ºC and 4 ºC, while free peroxidases lost their activity after 15-30 days. According to the results obtained, encapsulation of peroxidases offers the possibility of use in drug and pharmaceutical applications such as intestinal and colic protection against inflammation (Dahdouh et al., 2021).

CHAPTER 4

PEANUT AND CASHEW NUT BUTTER

4.1 Peanut

Peanut (*Arachis hypogaea L.)* is a food in the legume's family, which has high vitamin and mineral values (vitamins B and E, niacin, calcium and flavonoids) and is suitable for diet with 13% fiber (Table 4.1). It is also rich in oil (approximately 45%) and protein (approximately 24%) and is in oil seeds. Peanuts are widely used in the food industry, such as in the production of peanut butter, confectionery, roasted snacks, soups and meat products as a diluent. There are many varieties of peanuts that differ in grain size, shape and taste. There are three leading countries in peanut production and export in the world, namely China (45%), India (16%) and the USA (5%) (USDA, 2015). It is widely consumed with its high nutrient content in poor countries such as Africa and regions with harsh weather conditions such as Antarctica (Guimon and Guimon, 2012). Peanuts are also a functional food. It is antioxidant due to the content of vitamin E, ferulic acid, resveratrol and flavonoid, arginine (Geulein, 2010). Thanks to its coenzyme Q10 content, it has been recommended as a supportive supplement in the treatment of oxygen deficiencies in clogged vessels.

Table 4.1 Characteristics of Peanut from Turkomp (for 100 g peanut)

COMPONENTS	UNIT	AVERAGE
Energy	kcal	570
Water	g	5.77
Ash	g	2.29
Protein	g	23.51
Fat	g	45.42
Carbohydrate	g	10.49
Iron, Fe	mg	2.30
Phosphorus, P	mg	411

(Cont. on next page)

(Cont. of Table 4.1)

Calcium, Ca	**mg**	**62**
Magnesium, Mg	mg	189
Potassium, K	mg	677
Sodium, Na	mg	14
Zinc, Zn	mg	3.18
Thiamine	mg	0.667
Niacin	mg	1.735
Vitamin E	IU	12.31
Alfa-tocopherol	mg	8.26
Tryptophan	mg	330
Fiber	g	12.54
Arginine	mg	1182

Peanut butter is defined as the formation of a concentrated suspension by dispersing solid peanut particles in the oil phase (Co and Marangoni, 2012). One of the biggest problems with peanut butters in the food industry is oil oxidation. Fat oxidation accelerates contamination, impairs taste and odor, and reduces shelf life. In addition, the cleanness of the materials used during the production of peanut butter, the storage temperature and packaging conditions are also important for the shelf life. Peanut butters may contain 6-7% salt, sugar, oil and stabilizer additives due to the high costs in the food industry (Mohd Rozalli et al., 2016). Peanut butters are a highly preferred snack, especially by the American people. USA, Netherlands, China and Canada are the countries where peanut butter is exported the most.

Peanut roasting is usually done using an oven. This stage plays a critical role in peanut butter production. Because this stage affects the color, smell, oxidative stability and moisture properties of peanut butter. In addition, the roasting process ensures the removal of unwanted and contamination-causing microorganisms.

4.2 Cashew nut

The healthy nutrition trend that has come to the fore in recent years bring along people to eat healthy foods in small portions in a certain order. With the formation of the diet, the concept of elevenses has emerged. Nuts, which are healthy and useful snacks for body functions, are often preferred for elevenses. Many studies have reported that nuts have positive effects on staying in the body index range, struggling with obesity and chronic diseases (Ros, 2010). Cashew (*Anacardium occidentale L.)* is a fruit that stands out with its high essential amino acids and ascorbic acid, rich in protein (21%) and oil (46%) (Table 4.2). It is widely consumed all over the world and is preferred as an appetizer, especially in children, with its rich mineral and vitamin content (Ogunsina et al., 2014). It has shown an important bioactivity in thyroid mechanism, regulation of stress level and cancer diseases (Kannamkumarath, Wrobel, Caruso, & Vonderheide, 2002). In addition, in the food industry, cashew flour has been recognized as a nutritious flour variety due to its essential amino acids, flavonoids and balanced protein content.

Table 4.2 FAO reference values of cashew nut

Amino acids	Peptide coated cashew nut g 100 g $^{-1}$ protein	Amino acid requirement* g 100 g $^{-1}$ protein
Threonine**	3.22	2.30
Histidine**	2.20	1.50
Valine**	4.82	3.90
Methionine + cystine**	1.09	2.20
Cystine**	0.71	0.6
Isoleucine**	3.58	3.00
Leucine**	7.02	5.90
Tryptofan**	5.46	0.60
Phenylalanine + Tyrosine**	4.60	3.80
Lysine**	3.17	4.50
Aspartic Acid	7.43	N/E
Glutamic Acid	24.91	N/E
Serine	5.31	N/E
Glycine	4.24	N/E
Tyrosine	3.01	N/E
Arginine	9.84	N/E
Proline	7.33	N/E
Alanine	3.36	N/E

4.3 Peanuts of Relationship with Diseases

4.3.1 Obesity

The high nutritional value of peanuts and the abundance of vitamins, minerals and phalavoids in it have suggested that it may have positive effects on obesity, which is the leading disease of recent times. The bioactive components in it prevent the formation of chronic diseases by preventing inflammation and oxidative stress. The average calorie of 100 grams of raw peanuts is 567 kilocalories (USDA, 2018). Therefore, half a handful or 15 raw peanuts meet the daily intake need (USDA, 2015). In a study, it was determined that peanut consumption increased the basal metabolic rate by 11% (Alper & Mattes, 2002).

In an observational study, the healthy eating indexes of the foods that 4751 male, 4572 female and 4939 child participants ate for two days were calculated. It was reported that protein, fat, vitamin E, calcium, zinc, manganese and iron intakes of 24% of the participants who consumed peanuts were significantly higher than the other participants ($p<0.05$). In addition, when the cholesterol levels in the blood of the participants were examined, the cholesterol levels of those who consumed peanuts were found lower than the others. When the body mass indexes of the participants were examined, it was seen that those who consumed peanuts were lower than those who did not (Griel et al., 2004).

In another study investigating the effect of peanut and peanut butter consumption on body fat absorption, adult individuals were allowed to consume daily within a balanced diet plan, and some individuals were given 70 g/day peanuts daily. After a seven- to nine-day research program, it was observed that the fat absorption in participants who consumed peanuts was significantly higher than participants who did not consume peanuts ($p<0.05$) (Traoret et al., 2008).

4.3.2 Cardiovascular Diseases

Peanuts are low in saturated fat and also keep cholesterol levels low as they are free of trans fats. Therefore, it is thought to be very useful for cardiovascular diseases (Arya, Salve, & Chauhan, 2016).

In a study conducted in Iran, half of 44 male participants with hypercholesterolemia were told to maintain their normal eating patterns, and the other half to consume 76 g of peanuts daily. As a result of the four-week observation, it was determined that the total cholesterol and low-density cholesterol (LDL) levels of the participants who consumed peanuts were significantly lower than the other group and their high-density cholesterol (HDL) levels were significantly higher than the other group ($p<0.05$) (Ghadimi et al., 2010).

In another study, participants were divided into three groups. The first group ate 500 g peanuts in addition to their normal diet without dieting, the second group both dieted and ate 500 g peanuts, and the third group ate only peanuts. At the end of the study, only a decrease was observed in the amount of plasma triglycerides and total cholesterol in the first group, while there was no change in the lipid values of the participants in the other groups (Lokko et al., 2007).

4.3.3 Mental Health Disorders

Peanuts have high vitamin E and niacin content. Because of these ingredients, it has been found to be effective in Alzheimer's disease and age-related memory decline (Morris, 2014). At the same time, due to its resveratrol content, it has been found that sufficient consumption is beneficial in Alzheimer's disease and neurologic regression (Chen, 2005).

In studies conducted on middle-aged participants for 12 weeks, participants also consumed peanuts in their daily diet, and after this period, positive effects were observed on participants' memory functions, cognitive functions, and arterial elasticity (Barbour et al., 2017).

In a study conducted with individuals aged 55 and over in China, it was stated that the cognitive functions of individuals who consumed 10 g or more of peanuts daily were 40% higher (Li & Shi, 2019).

CHAPTER 5

MATERIALS AND METHODS

5.1 Materials

The cashew nuts and peanuts were purchased from the local market. Cashew and peanut butters were produced in Selçuk University Food Engineering Department Grain Laboratory.

Lactic acid bacteria isolated from artisanal cheese, identified by phenotypic methods (morphology, physiological and biochemical tests) and genotypic methods (16S-ITS PCR - RFLP) and characterized as *Lactococcus lactis* (Bulut, 2003) was used as psychobiotic. *Lactococcus lactis* C19.1 strain was obtained from İzmir Institute of Technology (İYTE) Food Engineering Department Molecular Food Microbiology Laboratory culture collection (İYTE-FED-FMLCC).

In all processes, all chemicals used were from Sigma and Merck. Growth media of microorganisms; M17 medium (S.r.l. Viale Monza, Biolife, Italy, Catalogue number: 4012782)

In microencapsulation process, $CaCl_2$ (Applichem, Germany, Catalogue number: 141221.1210) was used as droplet hardening agent. Whey protein isolate and soybean lecithin were used from Alfasol (Turkey). Sunflower oil was obtained from a local market Migros.

5.2 Methods

General outline of all methodological stages are shown in Figure 5.1. Firstly, First, whey protein and xylan were dissolved for 3 hours separately and whey protein was denatured. A coating solution containing whey protein, xylan and psychobiotics (*Lactococcus lactis* C19.1) was prepared. Cashew nut and peanuts were crushed in a laboratory food processor and were produced butter. After, this solution and peanut and

cashew nut butter were combined. In the third part, peanut butter and cashew nut butter samples were stored at 25°C. And then, samples analysis were started. The viability of psychobiotics before and after passing through the simulated gastrointestinal tract were analyzed. Finally, physicochemical (pH measurement, titration acidity, color, etc.), microbiological, *in vitro* (simulated mouth (SSF), stomach (SGF), and intestine (SIF)) and sensorial (oilness, taste, flavor, spreadability, etc.) analyzes of cashew nut and peanut butter samples were performed.

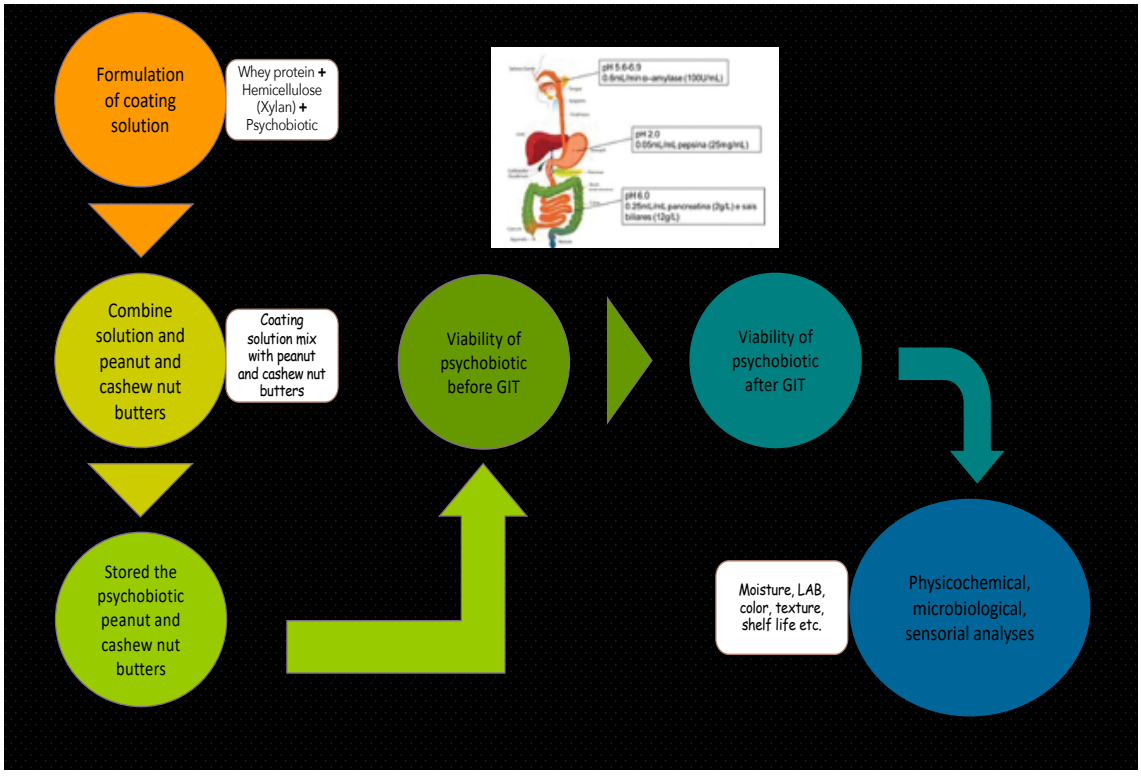

Figure 5.1 Methodologies applied at different stages of samples

5.2.1 Activation of Psychobiotic Bacteria

The psychobiotic property of the *Lactococcus lactis* C19.1 strain used in this thesis study, has been determined by HPLC method, GABA concentration was measured as 79.8 mg/L with the studies conducted within the framework of a continuing research project entitled as "Development of a Functional Whey Beverage; Enriched of Angiotensin-I Converting Enzyme (ACE)-Inhibitory Peptides and Gamma-Amino Butyric Acid (GABA); with Lactic Acid Bacteria Isolated from Artisanal Fermented Foods of Turkey" (Project No: TUBITAK-TOVAG 119O112), 01/09/2019-continue).

For the microencapsulation of psychobiotic bacteria, the emulsion method was used by making necessary adjustments on Çabuk and Harsa, (2015). First of all, resuscitation was carried out as a result of incubation of psychobiotic *Lactococcus lactis* C19.1 inoculated at a rate of 1% in 10 ml liquid M17 medium at 37 °C for 24 hours. The second inoculation process was carried out from the resuscitated bacteria to 10 ml liquid M17 medium at the rate of 1% and incubated at 37 °C for 24 hours. At the end of the incubation, the microorganisms were centrifuged (10000 rpm, 4°C, 15 min). After the liquid part remaining in the centrifuge tubes after centrifugation was poured, the collapsed parts were stored at 4 °C to be used in the microencapsulation process.

5.2.2 Microencapsulation of Psychobiotic Bacteria

Microencapsulation method was presented in Figure 5.2. Microcapsules were prepared by whey protein-xylan complex. In order to completely dissolve the whey protein (9%, w/v) prepared in pure water, mixing process was applied at 180 rpm at 4 °C for 3 hours. Whey protein solution was incubated at 80 ºC for 30 minutes in a water bath for denaturation. After the whey protein is denatured, it is left at room temperature for a while and cooled. The xylan (9% w/v) taken in a beaker was stirred for 3 hours on a magnetic stirrer to ensure complete dissolution in distilled water. Then, xylan (9%) was added to the solution and stirred in a magnetic stirrer for 30 minutes. At the end of 30 minutes, activated psychobiotic microorganism (10^{10} CFU/g) was added to the whey protein-xylan mixture.

To prepare microcapsules, 60 mL sunflower oil were mixed by whey protein-xylan 40mL mixture containing 10^{10} CFU/ml psychobiotic bacteria and homogenizated by Ultra Turrax (Ultra Turrax, model T25, Janke & Kunkel, IKA Labortechnik, Staufen, Germany) at 3500 rpm for 5 minutes. In the second step, 100 ml of $CaCl_2$ (0.1M) (Applichem, Germany) was dropped into the prepared emulsion (100 drops/minute) and homogenizing was continued for 2 minutes at 3500 rpm with Ultra Turrax homogenizer. After the microcapsule formation, this slurry was shaken for 30 minutes at 160 rpm for hardening of the microcapsules in the orbital shaker. To separate the hardened microspheres, the mixture was centrifuged at 5000 rpm for 1 hour and the microspheres

obtained by separating the liquid and oil from the top and stored at 4 °C in a screw cap glass bottle for analysis.

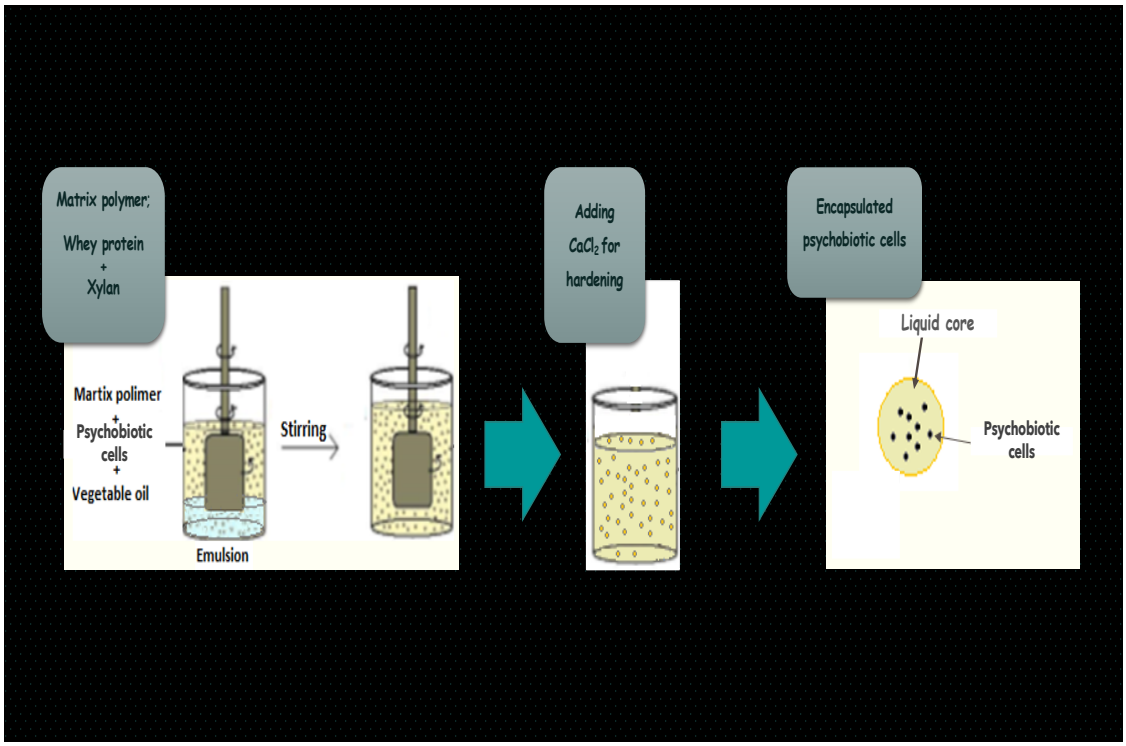

Figure 5.2 Microencapsulation and other analysis chart of samples

5.2.3 Production of Peanut Butters and Impregnation

Raw cashews and peanuts were obtained from the local market. 200 grams of each was taken. Raw cashews and peanuts were roasted in at 180 °C for 10 min using a laboratory digital electrical oven (sensitivity 1°C) (Kelinvestgroup, 2022). Roasted cashews and peanuts were thoroughly chopped and crushed with a laboratory food processor. To adjust the consistency suitable, 22% coconut oil was added to the cashew nut butter and 13% to the peanut butter. At the last stage, butter samples were divided into two as 100 grams.

Psychobiotics were added directly to half of the samples, and encapsulated psychobiotics were added to the other half. Each 100 grams was divided into 50 grams as the 1st replication sample and the 2nd replication sample. As a result, eight samples were produced, including two cashew nut butter samples and two peanut butter samples to which the psychobiotic was added directly, two cashew nut butter and two peanut butter

samples to which the psychobiotic was added by encapsulation. Produced samples were stored under sterile conditions at 25 °C.

5.2.4 Microencapsulation Efficiency

In order to count the bacteria entrapped in the microcapsule, the microcapsules must first be broken down. For this, microcapsules in peptone liquid were diluted at a ratio of 1/10. After taking samples from appropriate dilutions, petri dishes inoculated with the pour plate method were incubated at 37 °C for 48 hours under anaerobic conditions with using anaerobic kit (Thermo ScientificTM Oxoid AnaeroGen, England).

Encapsulation efficiency (EE%) was calculated according to the equation proposal by Rajam & Anandharamakrishnan (2015):

$$(\text{EE }\%) = \frac{N}{No} \text{ x } 100$$

Where N_o represent the number of viable *Lactococcus lactis* C19.1 after microencapsulation process and N represent the number of viable *Lactococcus lactis* C19.1 before microencapsulation process.

5.2.5 Storage Stability of Psychobiotics and Microbiological Analyses

In analysis, 1 gram of the products stored at 25°C was weighed, transferred to peptone water and shaken. The obtained suspensions were inoculated into M17 agar by the spread plate method by creating samples prepared in different dilutions using sterile peptone water. LAB colonies that developed in samples incubated at 37°C for 48 hours were counted. Results were determined in terms of total LAB. Samples taken at regular intervals throughout the shelf life were planted using the pour plate method as stated above, and then incubated and counted. Thus, the changes in LAB concentration during the shelf life and the viability of LAB in the control and final product were monitored.

5.2.6 *In vitro* Analysis

In vitro analyzes are performed to determine the extent to which the bacteria remain viable in the digestive tract on Çabuk & Harsa, (2015). We also performed this test separately for samples with encapsulated *Lactococcus lactis* C19.1 added and for non-

encapsulated *Lactococcus lactis* C19.1 added samples. Steps of *in vitro* anlaysis were shown in Figure 5.3.

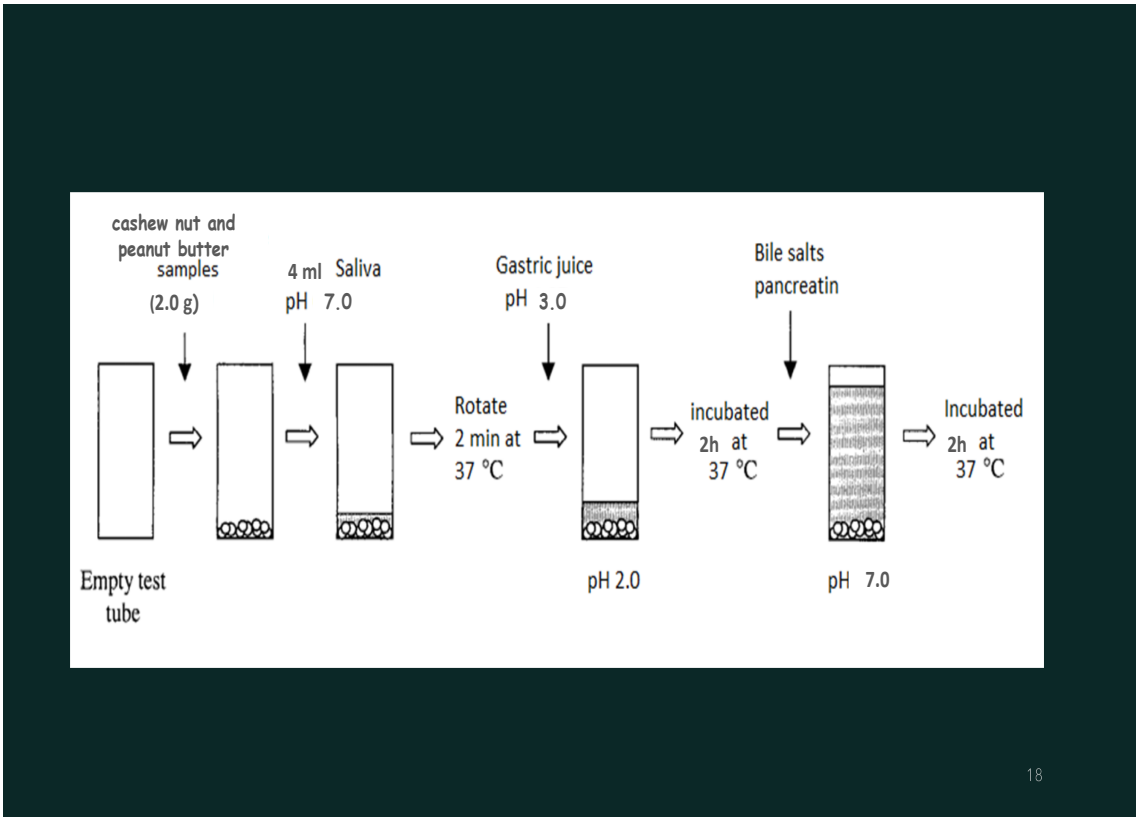

Figure 5.3 *In vitro* analysis chart of samples

First of all, three different solutions, mouth, stomach and intestine, and the enzymes to be added at these stages were prepared. At the same time, bile salt was prepared for the intestinal stage. The solutions are adjusted according to the pH data of the mouth, stomach and intestine.

In the first step, 2 g of each sample was weighed and the mouth stage was started. In the mouth, 3 ml of distilled water was added to 2 g samples and homogenized. Then, 2 µl of 0.3M $CaCl_2$ and 4 ml of SSF (simulated saliva fluid) (containing 250 mg of amylase enzyme) were added to the sample. To adjust the sample pH to 7.0, NaOH was added and this amount was recorded. After the total amount was completed to 10 ml, the samples were incubated at 37°C for 2 minutes. At the end of 2 minutes, one gram of each of the samples removed from the incubation was weighed and viability analysis was performed.

After the mouth stage, the stomach stage was passed. 8 ml of SGF (simulated gastric fluid) (containing 64 mg pepsin and 10 mg lipase enyzmes) and 5 µl $CaCl_2$ were added to the samples coming out of the mouth stage. In order to reduce the sample pH to 3.0, HCl was added and the amount added was recorded. The total amount of each sample was made up to 20 ml. For the stomach stage, each sample was incubated at 37°C for 2 hours. Then, viable count analysis was performed by taking 1 gram from each of the samples from the incubation. $NaHCO_3$ was added to the remaining samples to stop the activity of the pepsin enzyme.

In the last stage of the intestinal stage, 11 ml of SIF (simulated intestinal fluid) (containing 10 g of pancreatin enzyme), 25 ml of bile salt and 40 µl of $CaCl_2$ were added to the samples taken in the stomach stage. pH values of the samples were adjusted to 7.0, which is the pH of the gut microbiota, and the amount of NaOH added was recorded. The total amount of each sample was adjusted to be 40 ml and incubated at 37°C for 2 hours. Viable bacteria count analysis was performed by taking 1 gram from each of the incubated samples.

At each stage, the samples taken for viable counting were diluted and inoculated on M17 medium. Anaerobic kit was used for the media. The media were incubated at 37°C for 24 hours under anaerobic conditions and counted at the end of this period.

5.2.7 pH and Titration Acidity Measurement

The pH value of peanut and cashew nut butter samples were measured with a digital pH meter (Qualxtron®, Model 8010). The pH process was made by dissolving 2 g of butter in 10 ml of distilled water.

For titration, 2 g of each peanut and cashew nut butter samples were taken and dissolved in 10 ml of distilled water. Then, a few drops of phenolphthalein were added to each sample. NaOH was gradually added to the samples. The process was stopped when pink color formation was observed. Then, the amount of NaOH used was noted in volume as ml.

5.2.8 Color Measurement

The color of peanut and cashew nut butter samples were measured by the method reported by Mohd Rozalli et al. (2014). Konica Minolta colorimeter (model CR 410, Konica Minolta, Tokyo, Japan) was used for color measurement. The CIELAB system defined in the L*, a*, b* rectangle coordinates, where L * is measurement of the lightness, a * is measurement of the red-green and b * is measurement of the yellow blue.

5.2.9 Moisture Content and Water Activity

Briefly, 2 g of each peanut butter and cashew nut butter samples were weighed and spread in a dish and dried in a laboratory digital electrical oven at 105 °C for 6 hours (Mohd Rozalli et al., 2016). The products in closed containers were kept at room temperature for 30 minutes to reach ambient temperature. Moisture content was calculated according to their weight before and after entering the furnace, and the result was expressed as a percentage using the equation below.

$$Moisture\ content\ (\%) = \frac{M_{water}}{Msolid} \text{ x } 100$$

M_{water} refers to weight loss of sample after drying process (g) and M_{solid} refers to initial weight of sample before drying process (g).

5.2.10 Sensory Evaluation

Sensory evaluation was realized with the participation of 11 people, consisting of Selçuk University, Food Engineering Department students and staff. Evaluation took place in two sessions, for butters with encapsulated psychobiotic added in the morning, and butters with non-encapsulated psychobiotic added in the afternoon. The panelists were presented with a plate on which three-digits random numbers were coded on these samples, a tasting spoon, a glass of water, a napkin, a pen and an evaluation paper. A 9-point hedonic scale was used for evaluation (Giboreau et al., 2007). 4 grams of each sample were weighed and placed in the plate. Panelists evaluate from 1 = dislike extremely to 9 = like extremely.

5.2.11 Statistical Analyses

All experiments were performed combination of samples and parallel samples. Results were expressed with standard deviations. The data obtained was analyze using Minitab 2017 software and Excel365. Variance analysis (ANOVA) test, t-test and Z-test were performed in Minitab 2017 for the differences between the cashew nut and peanut butters formulations.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Stability and Viability of *Lactococcus lactis* C19.1 during *In Vitro* Digestion of Nut Butters

In vitro digestion analysis of the samples were carried out through a simulated gastrointestinal system in the laboratory environment. As stated in the methodology section, one gram each was taken from the samples after the mouth, stomach and intestine stages consequently and the viability of *Lactococcus lactis* C19.1 strain was counted. The results were expressed in Table 6.1. In addition, the graphs created to compare the results in the mouth, stomach and intestine stages were presented in Figure 6.1, 6.2, 6.3. As mentioned in the method section, when 10^{10} CFU/g bacteria were added to 100 grams of prepared samples, the bacterial population decreased to 10^{8} CFU/g. So the initial viability is 10^{8} CFU/g in all samples. Also, microencapsulation efficiency is 80 %. In Table 6.1, an approximately 3 logarithmic decrease was observed in FPB and FCB samples containing free cell psychobiotics. At the same time, since their final viability values remained below the literature value (Food and Health Agricultural Organization, 2002) of 10^{6} CFU/g, they lost their properties as psychobiotic products. A logarithmic decrease of 1.643 was observed in the CCB sample containing encapsulated psychobiotics and 0.733 in the CPB sample. According to the literature value (Food and Health Agricultural Organization, 2002), both products preserved their characteristics of being probiotic products. However, the peanut butter matrix in the CPB sample preserved more viable cells under simulated gastrointestinal conditions. This may be because the peanut butter matrix has more oil content and this oily phase may contribute as an emulsion type of effect that reduces contact with the external environment, resulting in less viable cell loss.

There have been limited number of literature research related to psychobiotic potential of functional food products. However, no research and none of the commercial foodstuff have been found so far about peanut and cashew butter products with probiotics having psychobiotic properties; therefore an original approach was designated within this

thesis study. Nevertheless, the effect of peanut butter matrix was examined for the viability of probiotics during their passage through the gastrointestinal tract, it was shown that peanut butter matrices could preserve probiotics in simulated gastrointestinal conditions (Klu & Chen, 2015). In this study, a logarithmic decrease of 2.14 in full-fat peanut butter and 2.13 in reduced-fat peanut butter was observed (Klu & Chen, 2015). Again in the same study, it was proven that more bacterial inactivation occurred under pH 2.0 conditions compared to pH 4.0 and 3.0. During the 6 hour simulated gastrointestinal transit, the greatest loss of viability was found in *Lactobacillus*, followed by *Bifidobacteria*. *Streptococcus/Lactococcus* had the least viability loss among the different probiotics used in the study (Klu & Chen, 2015). It was proven that *Lactococcus lactis* C19.1 strain used in the present thesis study has been the correct choice according to the findings of this article mentioned above. In addition, it was observed that 0.733 and 1.643 logarithmic reductions in CCB and CPB samples were determined, respectively, when these samples were produced in the laboratory bench scale within the framework of our thesis; there have been a significant difference in terms of viabilty values of probiotics as with 2.14 and 2.13 logarithmic reductions when compared with the data found by Klu and Chen, (2015). When both of the studies were compared, the nutty matrix used in our study was found to be much more compatible for *Lactococcus lactis* strain than the other study, and besides the encapsulation process used in our study was observed to be much more efficient, preservative and stable than the the one in the literature.

Table 6.1 Invitro analysis data of samples

	CCB	**CPB**	**FCB**	**FPB**
MOUTH	7.316 ± 0.153^{A}	7.522 ± 0.208^{A}	7.460 ± 0.028^{A}	7.505 ± 0.021^{A}
STOMACH	7.798 ± 0.374^{B}	6.957 ± 0.266^{B}	5.553 ± 0.098^{A}	6.012 ± 0.144^{A}
INTESTINE	6.357 ± 0.067^{B}	7.267 ± 0.114^{B}	5.167 ± 0.010^{A}	5.218 ± 0.036^{A}

Results are shown as means ± standart deviation. ($p<0.05$)

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added

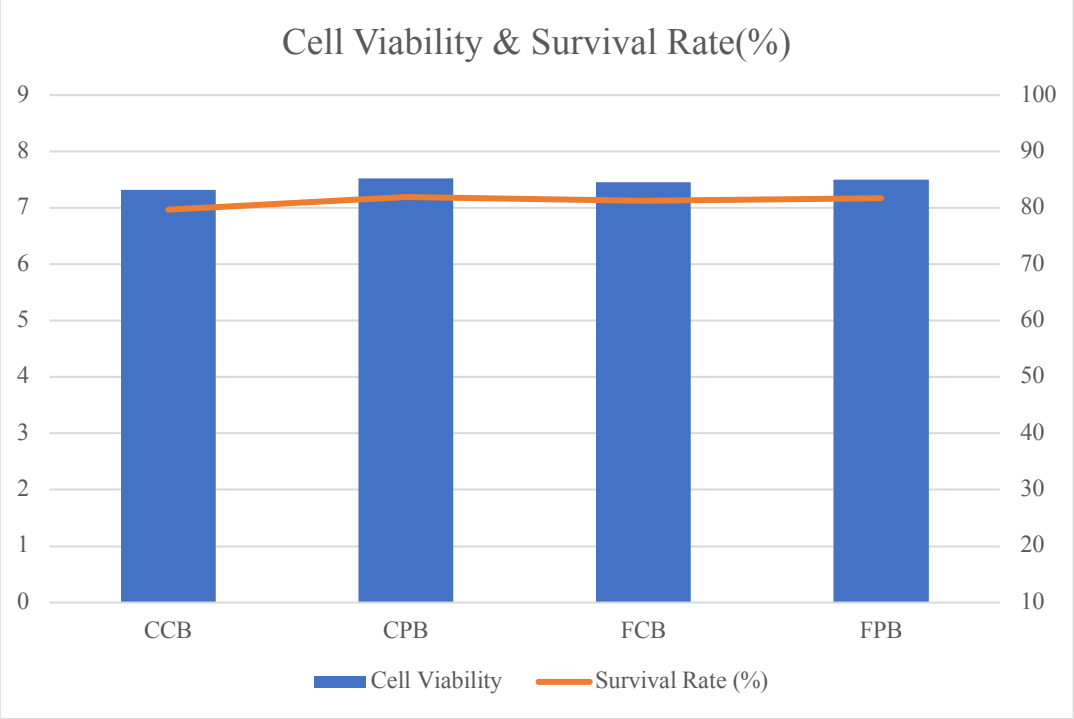

Figure 6.1 Cell Viability versus Survival Rate curve of free-cell and encapsulated samples in mouth (SSF).

In the simulated oral environment, the pH was 7.0, there has not been a corrosive pH environment created. Oral fluid saliva and enzymes worked in the mouth environment. Therefore, significant differences was not being observed among the samples in Figure 6.1. Even so, if necessary to compare the samples, CCB sample had 7.522 logarithmic value completed with maximum live cells. Two peanut butter samples, CCB and FCB, with logarithmic values of 7.522 and 7.505, respectively, completed this stage with the highest viable cell numbers. According to this result, it was observed that peanut butter matrix was more protective in simulated mouth path than cashew nut butter matrix since peanut butter samples might have more oily structure in comparison with cashew nut butter samples and/or the samples of peanut butter. This could be attributed to a more viscous structure of the peanut matrix after the production stage, thus keeping the psychobiotics more stable. Previous studies have also indicated the importance of the fat composition being as a protective barrier for live cells of probiotics during the transit

stage of simulated gastrointestinal tract (Possemiers et al., 2010; Ranadheera et al., 2012), which supports the hypothesis of this thesis as from the difference between the samples.

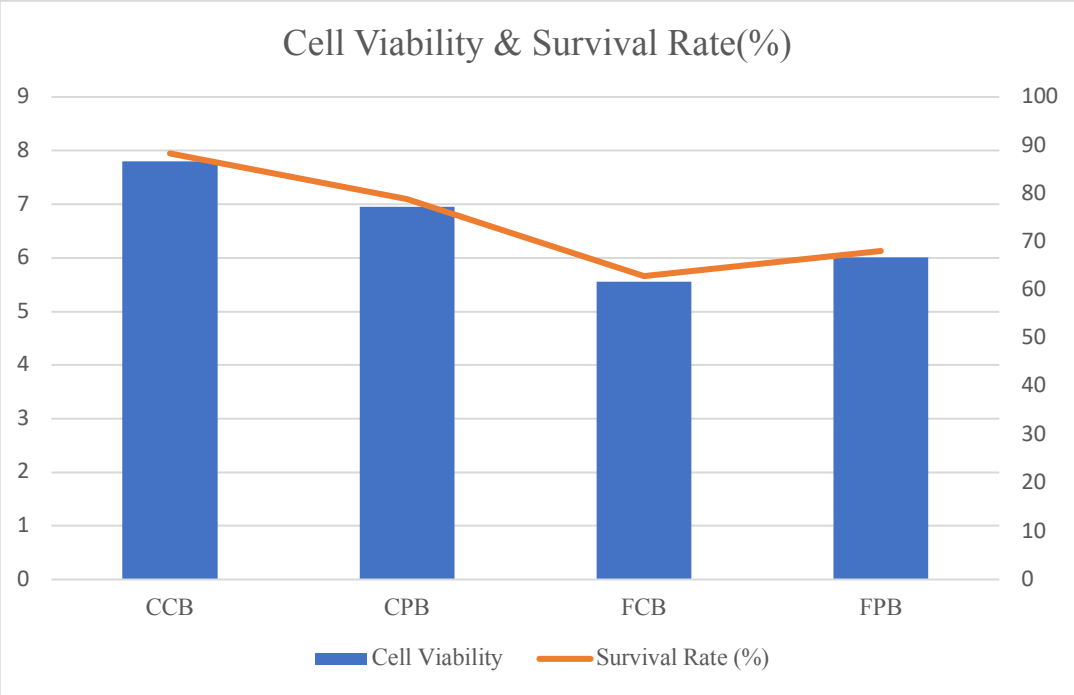

Figure 6.2 Cell Viability versus Survival Rate curve of free-cell and encapsulated samples in stomach (SGF).

Studies have shown that exposure of probiotic cells to gastric enzymes and acidic conditions of the stomach had a negative effect on probiotic viability, and probiotic cells were mostly inactivated at gastric pH conditions (Klu & Chen, 2015; Bove et al., 2013). Again in the same study, it was proven that more bacterial inactivation occurred under pH 2.0 conditions compared to pH 4.0 and 3.0. In Figure 6.2, the number of viable cells after samples exposure to gastric enviroment was presented as logarithmic. According to the graph, the most viable cell loss was seen in FCB and FPB samples containing free cell psychobiotics. A significant difference was observed between these samples and the CCB and CPB samples containing encapsulated psychobiotics. To compare these samples, the CCB sample had the highest viable cell count with a logarithmic value of 7.798. Based on this value, it has been observed that cashew nut butter matrix played a more protective role in gastric stomach conditions. This may be because cashew nut butter had a lower pH than peanut butter and its pH was closer to lower pH values. Thus, it did not undergo a sudden pH change as much as peanut butter.

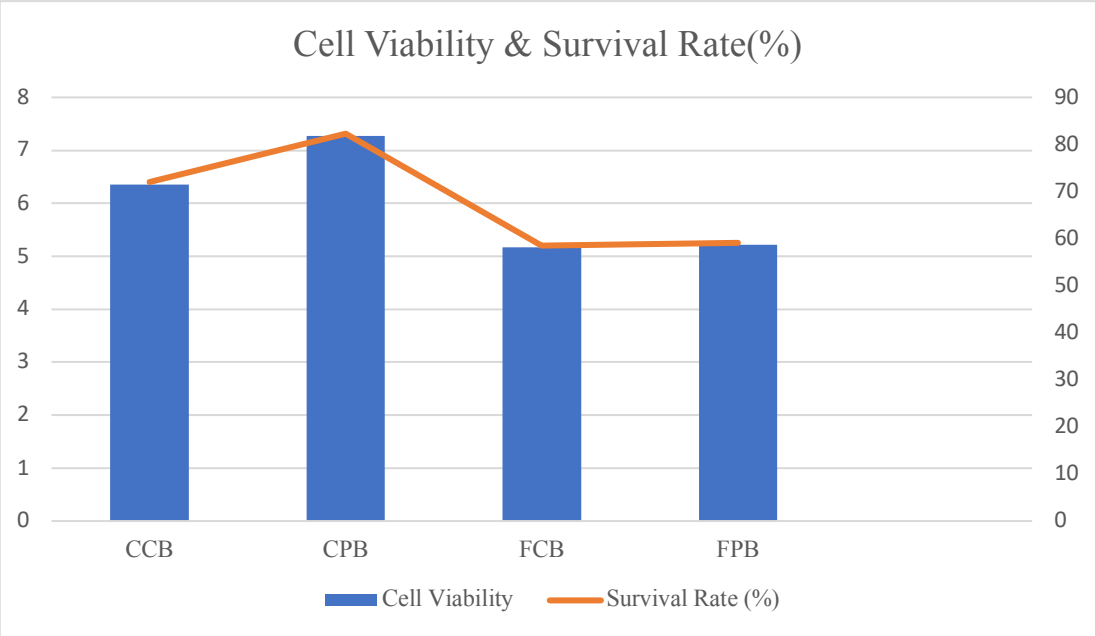

Figure 6.3 Cell Viability versus Survival Rate curve of free-cell and encapsulated samples in intestine (SIF).

The graph in Figure 6.3 shows the psychobiotic cell count in the samples recovered from the simulated intestinal environment. According to the results obtained, products containing encapsulated psychobiotics have the highest viable cell count. With these results, the protection of encapsulation on psychobiotic bacteria has been proven. The capsule minimized the bacteria's exposure to harsh external factors in the simulated gastrointestinal tract. While CCB has the highest number of viable psychobiotic cells in the previous stage, CPB has the highest number of viable psychobiotic cells at this stage. In the intestinal stage, the number of viable cells of CPB increased from 6.957 to 7.267 logarithmic values, while that of CCB decreased from 7.798 to 6.357 logarithmic values. For CPB, it is possible that some semi-lethal injured cells are healed and recovered in the intestinal phase (Klu & Chen, 2015). In this way, CPB has come to the fore as the highest psychobiotic content product. FCB and FPB, which are samples containing free cell psychobiotics, remained below the standard of being psychobiotic products in the literature and lost these properties.

The survival of encapsulated probiotics depends on the concentration of polymer used, capsule size, composition, etc. depends on a number of factors such as Dianawati et al. (2012) reported that sugar alcohols such as sorbitol and mannitol provide protection to probiotics by interacting with the polar region of the phospholipid bilayer. It has been

observed that when milk proteins were used together with alginate as encapsulation medium, the highly dense gel formed provides a suitable matrix for probiotics. The pH inside the gel matrix will be higher than in the external environment, thereby protecting the probiotics (Heidebach et al., 2009). In a previous study by Guérin et al. (2003) using a combination of alginate, pectin, and whey protein to encapsulate Bifidobacterium cells, there was a significant improvement in the survival of probiotics. In this thesis, a combination of whey and xylan was used as the capsule material, and it seems to be the right choice. According to the results obtained, CCB and CPB which containing encapsulated psychobiotics preserved their characteristics of being psychobiotic products property, but FCB and FPB which containing free cell psychobiotics couldn't preserve this property.

In a study on the addition of *Lactobacillus acidophilus* La-05 strain to goat ricotta cheese spread, the initial bacterial count was 7 log CFU in all products in *in vitro* analysis performed with products to which all probiotic formulations were added (Lopes et al., 2021). After the ileum part of the simulated gastrointestinal tract, 5.31 ± 0.03 log CFU bacteria were counted in the product containing free-cell (FREE), and 6.09 ± 0.08 log CFU bacteria were counted in the product containing encapsulated probiotics (AM) (Lopes et al., 2021). Bacterial counts were 5.167±0.010 log CFU in FCB and 5.218±0.036 log CFU in FPB after passage through the intestinal part. Compared with the FREE product, the bacterial counts of FCB and FPB had similar values. However, FREE has more bacterial count, suggesting that *Lactobacillus acidophilus* La-05 strain is more resistant to gastrointestinal conditions than *Lactococcus lactis* C19.1 strain used in this thesis. At the same time, this difference may be due to different matrices. Bacterial counts were 6.357±0.067 log CFU in CCB and 7.267±0.114 log CFU in CPB after intestine part of *in vitro* analysis. CCB and CPB samples have more bacterial count than AM. The reason for this difference suggests that the encapsulation method used for *Lactococcus lactis* C19.1 is more efficient than the encapsulation method used for *Lactobacillus acidophilus* La-05. In addition, the reason for this difference between bacterial counts can be thought to be cashew nut and peanut butter matrices are more protective than goat ricotta cheese matrix.

In other study, examining the effect of encapsulation on the viability of probiotics, encapsulated and free-cell probiotics were tested with *in vitro*. *Lactococcus lactis* CM22

was used as probiotic bacteria. According to the data obtained as a result of the tests, the survival rate of the encapsulated probiotic was found to be higher than that of free cells (Nivya & Nampoothiri, 2015). In general, when the graphs in three stages were examined, it is seen that similar results were obtained in this thesis. Encapsulation gives bacterial cells a greater chance of survival against difficult conditions in the simulated gastrointestinal tract. By this way, CPB and CCB samples preserve their properties as psychobiotic products.

6.2 Microbiological Analyses

For labeling a product as probiotic, it must contain at least 10^6 CFU/ml of live bacteria (Food and Health Agricultural Organization, 2002). This value is considered when determining the shelf life of a sample. When the viability of bacterial cell decreased to a value below 10^6 CFU/ml live bacteria, the enumaration was stopped and the time elapsed until this time was considered as shelf life. In this thesis, shelf life of samples was determined by monitoring the viability of pyshobiotic cell of four samples, two of which were cashew nut butters FCB, CCB and two of them peanut butters FPB, CPB. Psychobiotic cell counts of samples were made once every five days. Samples were stored at 25°C.

Table 6.2 Cell survival of free and microencapsulated *L. lactis* C19.1 in peanut and cashew nut butters for shelf life

TIME (Day)	CCB	CPB	FCB	FPB
0	9.181 ± 0.43^{abA}	8.830 ± 0.42^{abA}	7.903 ± 0.43^{cB}	7.670 ± 0.32^{cB}
5	8.231 ± 0.11^{aB}	8.136 ± 0.29^{aB}	7.420 ± 0.08^{aA}	7.266 ± 0.07^{aA}
10	8.122 ± 0.02^{aB}	7.322 ± 0.30^{aB}	7.455 ± 0.03^{aA}	7.305 ± 0.03^{aA}
15	8.195 ± 0.10^{bA}	7.535 ± 0.16^{bA}	7.222 ± 0.03^{cB}	7.12 ± 0.02^{cB}
20	7.905 ± 0.06^{abA}	7490 ± 0.07^{abA}	7.175 ± 0.01^{bA}	7.155 ± 0.01^{bA}
25	7.745 ± 0.06^{abA}	7.453 ± 0.03^{abA}	6.360 ± 0.03^{dB}	6.160 ± 0.05^{dB}
30	7.557 ± 0.03^{b}	7.419 ± 0.04^{b}		
35	7.35 ± 0.05^{ab}	7.358 ± 0.05^{ab}		
45	6.465 ± 0.07^{b}	6.303 ± 0.09^{b}		

Means refers to different small letters (a,b) show significant differences on the same column and means refers to different capital letters (A,B,C) show significant differences on the same line ($P < 0.05$).

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added,

As seen in Table 6.2, the shelf life of CCB and CPB samples was 45 days, and the shelf life of FCB and FPB samples was 25 days. The difference in days was of course due to encapsulation. Encapsulating the psychobiotic bacteria and adding cashew and peanut butter protected the bacteria by creating a barrier against external factors. As seen in Figure 6.4, a gradual decrease was observed in viable cell counts in samples, instead of a sudden decrease. Because the bacterial cells did not contact with the external environment directly. The oil phase in all samples acted as a barier for the bacteria to meet the harsh conditions in the external environment. At the same time, in CCB and CPB, which were the samples containing encapsulated psychobiotics, the capsule protected the bacterial cells against the external environment.

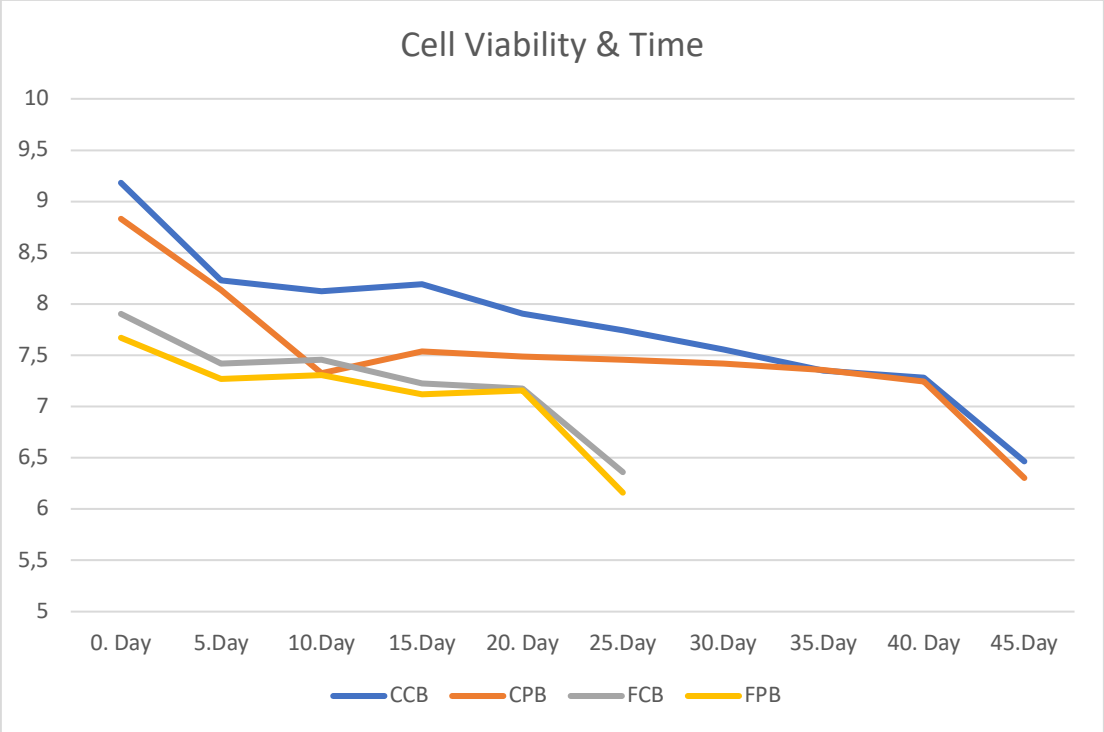

Figure 6.4 Graph showing the variation of cell viability (log) over time

In a study also described earlier on the addition of *Lactobacillus acidophilus* La-05 strain to goat ricotta cheese spread, similar *L. acidophilus* counts ($p \geq 0.05$) were made on the 1st day of storage in the microbiological analyzes performed on the products to which all probiotic formulations were added (Lopes et al., 2021). Bacterial counts of products containing microencapsulated *L. acidophilus* resulted in higher probiotic survival during storage (6.88 log CFU/mL versus 7.18 at day 7, $p < 0.05$) (Lopes et al., 2021). In this thesis study, the bacterial count results of the samples at day 5 were CCB (8.231±0.11 log CFU/g), CPB (8.136±0.29 log CFU/g), FCB (7.420±0.08 lof CFU/g) and FPB (7.266±0.07 log CFU/g). CCB and CPB samples containing encapsulated probiotics had highest live cells of probiotics than FCB and FPB containing free cell probiotics. In addition, when compared with the study (Lopes et al., 2021), cashew nut and peanut butter matrices in this thesis were found to be a more suitable matrix for probiotics than goat ricotta cheese, according to bacterial counts. In particular, the bacterial counts of the CCB (8.231±0.11 log CFU/g) and CPB (8.136±0.29 log CFU/g) samples differed greatly from the study count of 7.18 log CFU/g and had a very high value.

In a study, *L. casei* was encapsulated with pectin-alginate mixture and added to low-fat yogurt matrix (Sandoval-Castilla et al., 2010). The sample was stored at 4°C and had a shelf life of 20 days (Sandoval-Castilla et al., 2010). In another study, a functional product was developed by adding probiotic bacteria *L. acidophilus* LA-14 to cereal bar. As a result of 6 weeks of observation, the viability of probiotic bacteria in the cereal bar was determined as 7.33 log CFU/g (Bastos et al., 2014). In a similar study, a cereal bar was used but covered with probiotic whey protein isolate (Bastos et al., 2014). The probiotic viability rate, which was 9.21 ± 0.03 log CFU/g at the beginning, decreased to approximately 8.80 ± 0.02 log CFU/g after 45 days (Bastos et al., 2014). As seen in the literature examples given, the shelf life of CCB and CPB samples containing encapsulated psychobiotics is 45 days, which is quite sufficient. However, the shelf life of the product can be further enhanced by using different packaging material.

6.3 pH and Titration Acidity of Psychobiotic Added Samples

The pH values of food products stored during shelf life change over time. If your product is a functional food with added probiotics, pH changes and titratable acidity values are much more important. The pH values at which each bacteria can grow and

survive are different. For example, the growth range of *Lactobacillus acidophilus* is 5.5 to 6.0, while the optimum pH range for the growth of *Bifidobacteria* is 6.0–7.0 (De Vuyst, 2000). Some *Lactobacilli* species tend to survive in fermented products where pH is between 3.7 and 4.3. In another study, the development and survival rates of probiotics in fermented sausage were observed. According to the results obtained, it was observed that the probiotic *L. rhamnosus* GG and E-97800 strains used were affected by the decreasing pH value of the sausage after the fermentation process (Kołozyn-Krajewskaa & Dolatowski, 2012). According to these examples, the use of probiotics in the food industry, which are resistant to environments with low pH values such as bile and acid stresses, is beneficial to the consumer (Park, So, & Heo, 1995).

pH and titration acidity values quite effective on the survival rates of probiotics in products during their storage (Mortazavian et al., 2010). For example, beverages with low pH values such as fruit juice and turnip juice were resistant matrix into which probiotics could survive (Lima, Garruti & Bruno, 2012). pH and titration acidity determinations were made for cashew nut butter samples FCB and CCB and peanut butter samples FPB and CPB at ten-day intervals. Determinations were maintained throughout the shelf life of the samples. The results obtained were shown in Table 6.3 and Table 6.4 According to literature articles, the pH range for cashew nut butter was 5.9-6.6, while the pH value for peanut butter was around 6.3 (He et al. 2013; Lima, Garruti & Bruno, 2012). As seen in Table 6.3, the pH values of cashew nut butter samples were higher than the pH values of peanut butter samples. The results obtained were found to be in line with the literature ranges. Significant difference was not found between the pH values of the samples. During the shelf life, bacterial viability decreased as the pH value decreased since the decrease in pH value caused high acidity and *Lactococcus lactis* was not resistant to low pH values. However, according to the results obtained, the encapsulated psychobiotics in the samples CCB and CPB showed higher stability to the acidic environment than the free-cell psychobiotics in samples FCB and FPB. In other words, the microcapsules prevented the psychobiotics from high acidity.

Table 6.3 pH values of samples

TIME (Day)	CCB	CPB	FCB	FPB
0	6.225±0.007^{A}	6.515±0.03^{A}	6.325±0.02^{A}	6.660±0.01^{A}
10	6.170±0.01^{A}	6.560±0.04^{A}	6.160±0.001^{A}	6.470±0.001^{A}
20	6.145±0.007^{A}	6.475±0.007^{A}	6.040±0.001^{A}	6.380±0.02^{A}
30	6.150±0.01^{A}	6.375±0.02^{A}		
45	6.065±0.02^{A}	6.270±0.08^{A}		

Results are shown as means ± standart deviation. ($p<0.05$)

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added

Table 6.4 NaOH amount added in the samples

TIME (Day)	CCB	CPB	FCB	FPB
0	1.775±0.32^{A}	1.500±0.01^{A}	2.000±0.01^{A}	1.400±0.01^{A}
10	1.800±0.28^{A}	1.650±0.07^{A}	2.050±0.21^{A}	1.500±0.01^{A}
20	1.875±0.32^{A}	1.675±0.09^{A}	2.000±0.01^{A}	1.600±0.01^{A}
30	2.400±0.56^{A}	1.900±0.14^{A}		
45	2.600±0.14^{A}	2.300±0.28^{A}		

Results are shown as means ± standart deviation. ($p<0.05$)

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added

General or specific acidity determinations are made in foods for many reasons. First of all, acidity determination is made in order to determine the functional properties and behaviors of food proteins. Secondly, acidity determinations can be made to determine the durability of some foodstuffs to storage. For example, the durability of pickles and sausages to storage is due to their low pH. In addition, the conformity of some foodstuffs to standards is registered with acidity determination. For example, it is undesirable for the acidity to be more than 0.1% in wines (Erciyes University, 2023). Likewise, it was undesirable for the ratio of free acids to be more than 1% in vegetable and animal oils, and less than 1% and more than 2% in pickles (Erciyes University, 2023). Again, the amount of acidity was found to be very important in meat, milk and some of their products. Peanut butter and cashew nut butter are food matrices having promising properties for psychobiotics, low moisture content, and satisfactory shelf life. With the addition of psychobiotics to these matrices, psychobiotic bacteria activity occurs in the matrices over time. As a result, the pH of the matrices decreases over time due to the psychobiotic activity. Table 6.4 presents the amounts of NaOH used for the determination of the titration acidity of the samples. NaOH values were measured during the shelf life of the samples. It was determined that the amount of NaOH used in the samples was inversely proportional to the pH values of the samples. As the pH values decreased, the amount of NaOH used increased. Because high volumes of NaOH had to be used to neutralize the pH values of the samples. Samples containing encapsulated psychobiotics used less NaOH amount as there was less pH drop. The microcapsule provided the preservation of psychobiotic viability.

6.4 Color Properties of Butters Samples

Peanut butter color is highly effective on aroma and flavor parameters as it is related to the degree of roasting (Abegaz & Kerr 2006). The color of the product depending on the degree of roasting is one of the main parameters in determining the quality of the product (Abegaz & Kerr 2006). Roasting also affects L*, an important color parameter. Reactions such as caramelization reactions, non-enzymatic browning, and phospholipid degradation in the roasting process can cause a decrease in the L* parameter in color. A

CIELab color scale was used to measure the degree of lightness (L), redness (+a) or greenness (-a) and yellowness (+b) or blueness (-b) of the films. Color analyzes of four samples were presented in Table 6.5. Cashew nut butter and peanut butter samples were shown in Figure 6.5. Two peanut butter samples, FPB and CPB, and two cashew nut butter samples, FCB and CCB, had similar color parameter values. Although all samples were roasted for the same time and degree in electrical oven, there were significant differences between them. First of all, when the same type of samples containing encapsulated psychobiotics and free cell psychobiotics were examined, it was determined that there was not significant difference between them ($p<0.05$). In other words, the microcapsule did not have a significant effect on the products color.

Table 6.5 Color measurements of samples

	CCB	**CPB**	**FCB**	**FPB**
L*	40.195 ± 0.12^{A}	53.970 ± 0.7^{B}	41.230 ± 0.24^{A}	53.816 ± 0.16^{B}
a*	8.318 ± 0.06^{B}	2.876 ± 0.07^{A}	8.370 ± 0.16^{B}	2.795 ± 0.05^{A}
b*	13.766 ± 0.33^{A}	18.510 ± 0.23^{B}	13.740 ± 0.39^{A}	18.780 ± 0.08^{B}

Results are shown as means ± standart deviation. ($p<0.05$)

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added

L* = degree of lightness

a* = redness or greenness

b* = yellowness or blueness

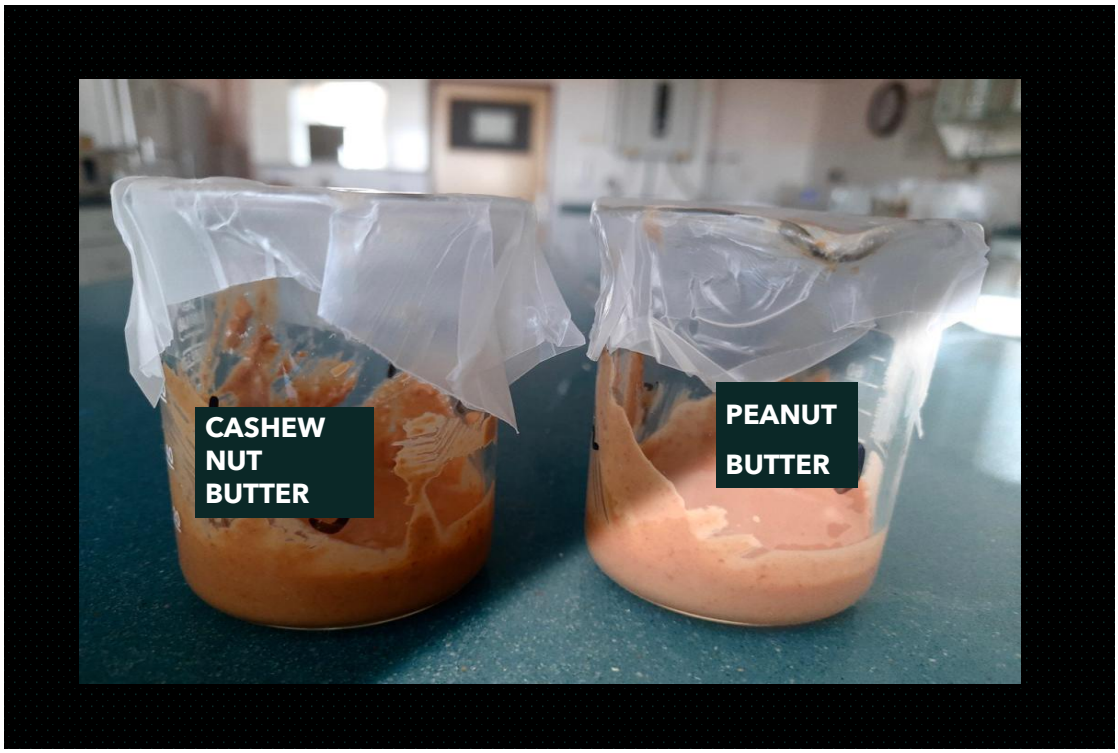

Figure 6.5 Peanut butter and cashew nut butter

In a study, four types of peanuts and peanut butter from different regions of China were produced and their color changes and other sensory properties were investigated by applying microwave (800 W at 5 minutes) to all of them (Degon et al., 2021). The L* value range was determined as 51-52 according to the control sample. According to the results obtained, the L* parameter values in four types of samples were determined as 51.08, 51.31, 47.67 and 51.40, respectively. According to the results, 1st, 2nd and 4th samples provided the appropriate range, while the 3rd sample was outside this range. From this point of view, there have been different color characteristics of peanuts and peanut butters even if they are produced from the same peanut source (Degon et al., 2021). Although the colors of raw peanut and cashew nut were close to each other, there were significant differences in the peanut butter and cashew nut butter samples after the roasting process. The L* value of CPB was 53.970 and the L* value of FPB was 53.816, which was very close to the L* value 51-52 in the literature. The L* value of CCB was 40.195 and the L* value of FCB is 41.230, which was found quite different from the literature L* value. The nutritional ingredients of cashew nut and peanut were not the same. They contain different amounts of protein and glucose. In addition, the fat ratios were also different. For these reasons, the rates of temperature affection were also different. According to Table 6.5, it was observed that peanut butter samples CPB and

FPB were more resistant to temperature than cashew nut butter samples FCB and CCB. For this reason, cashew nut butter samples CCB and FCB should be roasted at a lower temperature and time in order to avoid color differences.

6.5 Water Activity and Moisture Content

The a_w for commercial peanut butter was 0.29 (FDA, 2014). The addition of additives and stabilizer contributes to the higher a_w value of commercial peanut butter. Low and stable aw is suitable to prevent microbial growth during storage. In the study of Burnett et al. (2000), it was reported that the aw of regular peanut butter was higher than natural peanut butter, 0.29 versus 0.22. Only the storage time factor had a significant effect on the airiness of the natural peanut butter ($p<0.05$). Formulations containing free psychobiotics showed a reduction in a_w after 25 days of storage and formulations containing microencapsulated psychobiotics after 45 days of storage, possibly a balance between ambient humidity and product after opening. Low a_w food products are known to inhibit the growth of microorganisms, but they can increase oxidation reactions that are detrimental to the lipid matrix. Therefore, the oxidative stability of peanut butter formulations was evaluated.

Moisture content is one of the most important factors affecting the stability of the matrix. It has been observed that this value varies between 2.8 and 1.6 in the results obtained from free-cell and encapsulated samples. This value was found to be in accordance with the literature values (FDA, 2014).

Also, water activity (a_w) is sensitive to temperature. When examined in different food matrices, different effects of temperature were seen. Especially when looking at low moisture foods such as peanut butters, it was seen that the survival rate of the bacteria in the food matrix increases as the water activity decreases (Syamaladevi et al., 2016). However on the other hand, there is a direct correlation between water activity and food contamination in low moisture foods. In one study, *Salmonella* counts that could develop in the matrices of wheat flour and peanut butter were counted during their shelf life. According to the data obtained, there was less contamination in peanut butter than wheat flour and *Salmonella* was less developed (Syamaladevi et al., 2016). According to another

study, it has been observed that the lipid phase protected the bacteria in the food matrix against environmental factors in high-fat, low-moisture foods such as peanut butter and cashew nut butter (Yang et al., 2020).

6.6 Sensory Evaluation

Samples of CCB, CPB containing encapsulated psychobiotics and samples and samples of FPB, FCB containing free-cell psychobiotics were used for sensory evaluation. Trained 11 panelists were selected for the sensory test. Samples of CCB and CPB containing free-cell psychobiotics in the morning and FPB and FCB containing encapsulated psychobiotics in the afternoon were scored by the panelists. The materials and samples presented to the panelists in the sensory test were shown in Figure 6.6. The results obtained after analyzing samples scores of all panelists were presented in Table 6.6 and Figure 6.7. As seen in Table 6.6, there was not significant difference in the overall acceptability of the samples and the scores were close to each other. However, peanut butter which contains encapsulated psychobiotics CPB was the most popular sample compared to the others. This may be because cashew nut butter was a new product to consumers, but people were more familiar with peanut butter.

One of the most important features affecting the quality and consumer taste in cashew nut and peanut butter was its consistency and smooth structure (Tournier et al., 2007). In order to obtain a smooth structure, the grinding rate must be high. In a study, cashew nut butters with different grinding rates and different sizes of particles were presented to the consumer. The most favored samples were those with smaller particle size and more ground. As can be seen in Table 6.6, the spreadability of all samples was appreciated and accepted. All samples were subjected to the grinding process equally. Figure 6.6 shows the test environment and the materials presented.

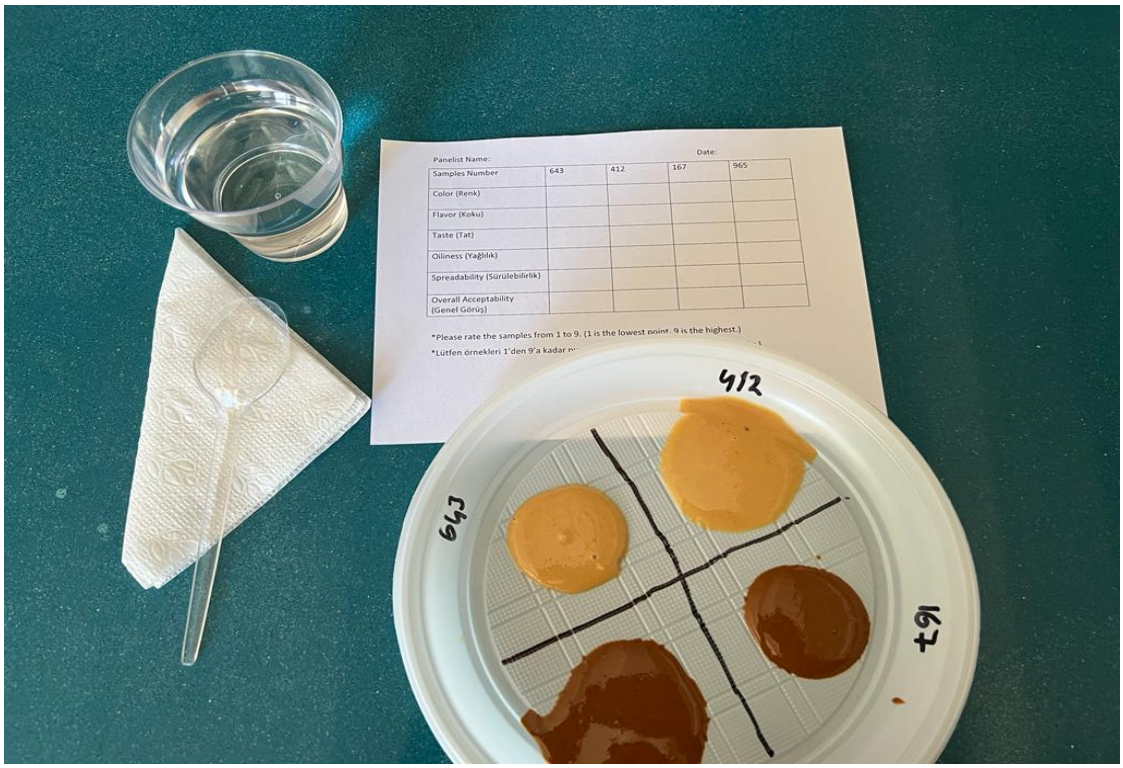

Figure 6.6 Sensory evaluation test picture

Table 6.6 Data of Sensory Test

	CCB	CPB	FCB	FPB
Color	7.409±1.272^{A}	7.863±1.250^{A}	7.454±1.250^{A}	7.318±1.501^{A}
Flavor	7.636±1.433^{A}	7.545±1.433^{A}	7.363±1.507^{A}	7.909±1.445^{A}
Taste	8.363±1.213^{AB}	7.454±1.809^{B}	8.272±0.934^{A}	8.363±1.572^{A}
Oiliness	8.090±1.044^{AB}	8.727±1.078^{A}	7.909±1.044^{B}	7.909±0.943^{B}
Spreadability	7.636±1.692^{A}	7.727±1.420^{A}	7.181±1.167^{A}	7.636±1.103^{A}
Overall Acceptability	7.454±0.934^{A}	7.909±1.206^{A}	7.272±0.786^{A}	7.363±0.943^{A}

Results are shown as means ± standart deviation. ($p<0.05$) (n=11)

CCB: cashew nut butter sample with encapsulated psychobiotic added

CPB: peanut butter sample with encapsulated psychobiotic added

FCB: cashew nut butter sample with free-cell psychobiotic added

FPB: peanut butter sample with free-cell psychobiotic added

In addition, for peanut butter and cashew nut butter samples, the oiliness ratio and the shine arising from this oiliness are important (Giboreau et al., 2007). The excess oiliness in butters upsets the stomachs of the consumers, the low oiliness leaves an acrid taste in the mouths of the consumers, and all these situations reduce the sales of the product. For this reason, the oil rate and consistency of the product should be just in balance. As seen in Table 6.6, all samples received a passing grade from the consumers and were scored similarly. However, peanut butter CCB, which contains encapsulated psychobiotics, was more popular than the others. The reason for this may be that peanut had a high amount of its own oil and this ratio of oiliness may be liked by the consumer.

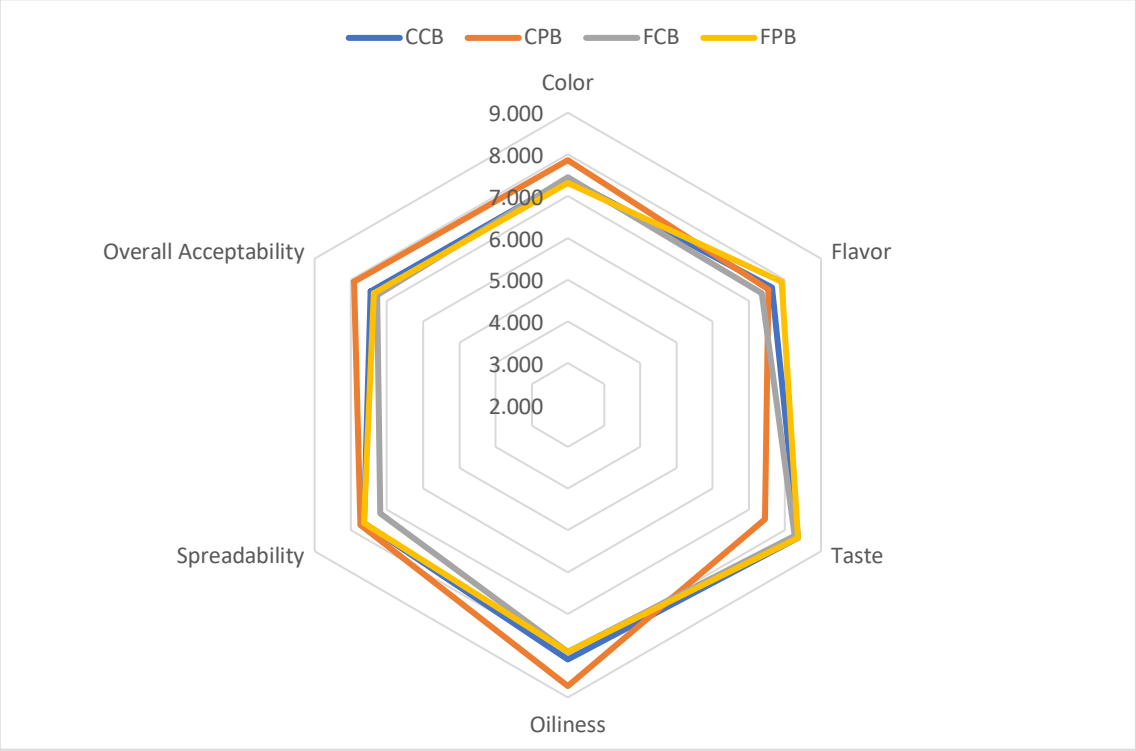

Figure 6.7 Radar diagram of sensory evaluation

In a study on the addition of *Lactobacillus acidophilus* La-05 strain to spreadable goat ricotta cheese, analyzes were made on the sensory effects of microencapsulation on

the product (Lopes et al., 2021). According to these analyses, goat cheeses containing microencapsulated bacteria had higher lactose content as well as lower lactic, acetic acid, and galactose during storage than goat cheeses containing free cell bacteria ($p<0.05$). Microencapsulation of *L. acidophilus* was thought to prevent the probiotic strain from interacting with the food matrix, hydrolyzing lactose, and producing lactic and acetic acids (Meira et al., 2015). The presence of galactose is associated with the appearance of off-flavors in products (Muelas et al., 2018), while the presence of acetic acid is associated with a sharp vinegar taste in products (Miranda et al., 2019). For this reason, low galactose and acetic acid concentrations in microencapsulated probiotic products are important in terms of sensory. In this thesis study, general acceptability of CCB and CPB products containing encapsulated *L. lactis* C19.1 (7.454±0.934 and 7.909±1.206) was higher than general acceptability of FCB and FPB products containing free cell (7.272±0.786 and 7.363±0.943), which can be associated with microencapsulation. On the other hand, according to the study by Lopes et al., (2021), the inclusion of free or microencapsulated *L. acidophilus* resulted in lower hardness and viscosity but higher spreadability and fluidity in the control product ($p<0.05$). The presence of probiotic cultures can increase the spreadability of products (Miranda et al., 2019), therefore, as seen in Table 6.6, the spreadability of CCB and CPB (7.636±1.692 and 7.727±1.420) and spreadability of FCB and FPB (7.181±1.167 and 7.636±1.103) were found close to each other; this may be the reason why high values were obtained.

Process of microcapsulation to peanut butter samples of CPB, FPB and cashew nut butter samples of CCB, FCB did not affect the sensorial qualities as shown in Table 6.6 and Figure 6.7. The taste and oiliness of peanut butter and cashew nut butter samples may be altered after adding the microcapsules, but all formulations showed a score of about 7.5, confirming some of their instrumental parameters. The encapsulated psychobiotic significantly affected the taste of peanut butter and cashew nut butter, but this did not adversely affect its acceptability. Looking at the overall acceptability, it was seen that all the butter samples received similar scores. Based on this, peanut butter which contains encapsulated psychobiotics CPB received the highest purchase and appreciation score. Cashew nut butter which contains encapsulated psychobiotics CCB followed this score. These results showed that CCB and CPB had an acceptable sensory properties were found suitable for peanut and cashew nut butter production.

CHAPTER 7

CONCLUSION

In this study, psychobiotics were added to peanut and cashew nut butters, which are consumed by many consumers in their daily life, to reduce the stress level of consumers and to make them feel more comfortable and peaceful in their daily lives. Peanut and cashew butters were selected, which can be determined as a excellent matrix for psychobiotics since they also exert supporting properties. *Lactococcus lactis* C19.1 strain with proven high GABA activity was used as a model psychobiotic bacteria. As a result of devoted studies and comparisons, the production of peanut and cashew paste, which had a shelf life of 45 days, was successfully completed by encapsulating psychobiotic bacteria with CCB had 6.465±0.07 CFU/g and CPB had 6.303±0.09 CFU/g. The adequate shelf life of the samples makes this product a very promising functional product. In the light of the information obtained from *in vitro* analyzes, it has been proven that the live cells survival in the intestine for CCB is 6.357±0.067 CFU/g and CPB7.267±0.114 CFU/g was above 10^6, and psychobiotic bacterial cells could reach through enrich the intestinal microbiota. In terms of better product quality attributes, since different colors of peanut butter and cashew butter were obtained, applying different roasting temperatures can be suggested. The products offered to the consumer's taste showed that they could find a place for themselves when they entered into the food market by getting acceptable grades with CCB (7.454±0.934), CPB (7.909±1.206), FCB (7.272±0.786) and FPB (7.363±0.943).

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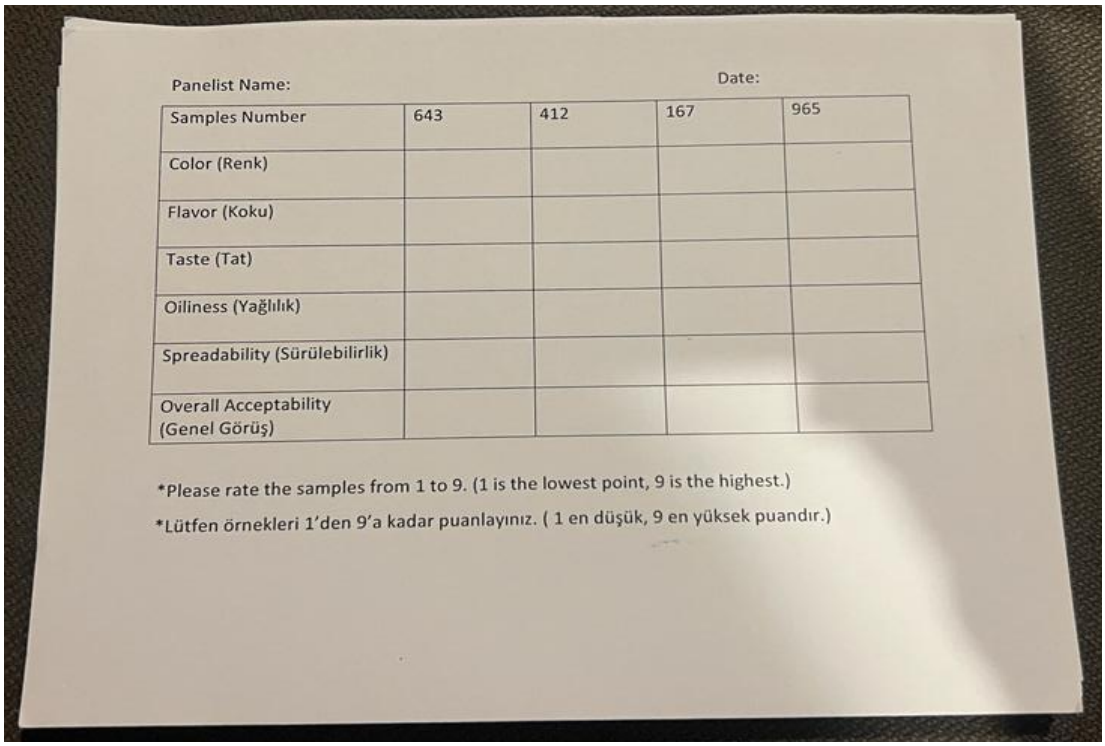
APPENDIX A

SENSORY ANALYSE TEST PAPER



Panelist Name: Date:

Samples Number	643	412	167	965
Color (Renk)				
Flavor (Koku)				
Taste (Tat)				
Oiliness (Yağlılık)				
Spreadability (Sürülebilirlik)				
Overall Acceptability (Genel Görüş)				

*Please rate the samples from 1 to 9. (1 is the lowest point, 9 is the highest.)

*Lütfen örnekleri 1'den 9'a kadar puanlayınız. (1 en düşük, 9 en yüksek puandır.)

Figure A.1. Sensory analyse test paper of peanut and cashew nut butter samples