DEVELOPMENT OF FERMENTED VEGAN FOOD FORMULATIONS WITH IMPROVED ANGIOTENSIN-I CONVERTING ENZYME INHIBITORY (ACE-I) ACTIVITY

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ABSTRACT

DEVELOPMENT OF FERMENTED VEGAN FOOD FORMULATIONS WITH IMPROVED ANGIOTENSIN I CONVERTING-ENZYME INHIBITORY (ACE-I) ACTIVITY

Veganism has become popular in recent years and the demand for vegan products is increasing, especially due to the positive effects of plant-based diets on health. Since veganism can be defined as a diet or lifestyle that does not support the consumption of any animal food, protein-rich legumes occupy a large place in the daily diet of vegans. The health-promoting potential of probiotics in various forms has been recognized for years, and one of these health-promoting properties is their antihypertensive effect. In this context, the aim of this thesis is to develop some vegan food formulations, such as vegan mayonnaise-based salad dressings prepared using chickpea aquafaba and plant-based milk alternative (PBMA) fermented using lactic acid bacteria (LAB), showing antihypertensive effect. Microbiological, and quality characteristics and antioxidant properties of the formulations were determined. In addition, proteolytic activity and angiotensin-I converting enzyme inhibitory (ACE-I) activity experiments were carried out to control the antihypertensive properties of salad dressings. As a result, there are 10^7 CFU/ml bacteria in the final products. The quality characteristics of the formulations were compared with the literature. Consequently, the ACE-I activity of PBMA was enhanced by LAB fermentation and vegan mayonnaise was enriched with these high-value ingredients.

ÖZET

ANJİYOTENSİN-I DÖNÜŞTÜRÜCÜ ENZİM İNHİBİTOR AKTİVESİ ARTIRILMIŞ FERMENTE VEGAN GIDA FORMÜLASYONLARININ GELİŞTİRİLMESİ

Veganlık son yıllarda popüler hale gelmiştir ve özellikle bitki bazlı diyetlerin sağlık üzerindeki olumlu etkileri nedeniyle vegan ürünlere olan talep de artmaktadır. Veganlık, herhangi bir hayvansal gıdanın tüketilmesini desteklemeyen bir beslenme ya da yaşam biçimi olarak tanımlanabileceğinden, veganların günlük beslenmelerinde protein açısından zengin baklagiller büyük yer tutmaktadır. Probiyotiklerin çeşitli şekillerde sağlığı geliştirme potansiyeli yıllardır bilinmektedir ve bu sağlığa yararlı özelliklerinden biri de antihipertansif etkisidir. Bu bağlamda, bu tezin amacı, nohut aquafabası kullanılarak hazırlanan vegan mayonez bazlı salata sosları ve laktik asit bakterileri (LAB) kullanılarak fermente edilmiş bitki bazlı süt alternatifi (PBMA) gibi antihipertansif etki gösteren bazı vegan gıda formülasyonları geliştirmektir. Formülasyonların mikrobiyolojik ve kalite karakteristikleri ve antioksidan özellikleri belirlenmiştir. Ayrıca, salata soslarının antihipertansif özelliğini kontrol etmek için proteolitik aktivite ve anjiyotensin-I dönüştürücü enzim inhibitör (ACE-I) aktivitesi deneyleri yapılmıştır. Sonuç olarak, nihai ürünlerde 10^7 KOB/ml bakteri bulunmaktadır. Formülasyonların kalite karakteristikleri literatürle karşılaştırılmıştır. Sonuçta, PBMA'nın ACE-I aktivitesi LAB fermantasyonu ile artırılmış ve vegan mayonez bu yüksek değerli bileşenlerle zenginleştirilmiştir.

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LIST OF ABBREVIATIONS

SD: Salad dressing

UPM: unpasteurized mayonnaise

PBMA: Plant-based milk alternative

F. PBMA: Fermented plant-based milk alternative

MB: YSL: mung bean and yellow split lentil

CP: Chickpea

MPBMA: mixture of mung bean, yellow split lentil, and chickpea milk alternative with mixing ratio (1:1:2) (v/v)

AQF: Aquafaba

LAB: Lactic acid bacteria

ACE: Angiotensin-I converting-enzyme

ACE-I: Angiotensin-I converting-enzyme inhibition

Ang I: Angiotensin I

Ang II: Angiotensin II

OPA: O-phthaldialdehyde

ESI: Emulsion stability index

EAI: Emulsifying activity index

WHO: World Health Organization

EFSA: European Food Safety Authority

CHAPTER 1

INTRODUCTION

1.1. Plant-Based Diet

In the 21st century, one of the main problems to be faced is to feed the increasing population with decreasing natural resources day by day. While scientific research is interested in healthiness and well-being correlated with balanced nutrition, development of new food products and searching of new natural components are increasing (Pimentel et al. 2021). In the meantime, food preferences of consumers focus more on functional foods which can provide healthiness and well-being with high nutritional values (Pontonio et al. 2020; Pimentel et al. 2021). In this context, it is an increasing trend not to consume animal derived products, therefore it can be said that there has been an increase in vegetarianism and/or veganism (Pimentel et al. 2021).

Veganism can be defined as a diet and a way of living that doesn't provide any food or some other products which come from animal origin such as animal origin clothes, cosmetic products, meat, honey, egg, or dairy products. It can also be named as plant-based diet or lifestyle (North et al. 2021). Even though veganism is a type of vegetarianism, it is considered separately (Akkan and Bozyığıt 2020). In the study of Saari et al. (2021), it was stated that 19%, 16%, 8%, 6%, and 5% of the respondents followed a vegetarian diet in the Asia-Pasific region, Africa/Middle East, Latin America, North America, and Europe, respectively in 2016. Besides that, a vegan diet was followed by 9%, 6%, 4% of the respondents in the Asia-Pasific region, Africa/Middle East, Latin America, respectively and 2% of the respondents both in North America and Europe (Saari et al. 2021). People can have multiple reasons such as sustainability concerns, animal welfare, ethics, and protection of personal health to be vegan (Akkan and Bozyığıt 2020). As a result of the Janssen et al. (2016) research about the consumer motivations for following a vegan diet, there are three dominant reasons: respondents made mention of animal welfare (89.7%), health and/or personal well-being (69.3%), and environmental reasons (46.8%). Basically, there are criticisms about unhealthiness to consume just plant

origin foods. However, this is not a situation that cannot be handled with a well-balanced diet program (Akkan and Bozyığıt 2020). Bakaloudi et al. (2021) concluded that the energy intakes are not below the reference nutrient intake even though those values are lower in veganism than common diet types. The consumption of macro nutrients is adequate except for the protein content. Glycemic index and lipid profiles are more convenient in a vegan diet. However, deficiencies of micronutrients, such as Vitamin B2, B3, B12, vitamin D, calcium, iodine, zinc, and selenium, can occur. According to results, nutrient inadequacies can be challenging but vegans have lower body mass index and the cancer incidence, overweight and obesity are lower in vegans in comparison with other types of diet. The diet can be arranged by taking into consideration potential deficiency risks.

The demand for food products, which does not contain meat, grew in the ratio of 987% in 2017 just in the United Kingdom (UK) (Pimentel et al. 2021). That same year, there were approximately 1.8 million people in Italy who declared to follow a plant-based diet, equaling three times the percentage of people choosing a vegan diet over their population compared to the previous year (Bedin et al. 2018). Besides that, non-dairy milk alternatives have become a multi-billion-dollar business over the years in the global market and are going to continue to grow according to the economic predictions (Pimentel et al. 2021). Based on the results of American market research platforms, which were given in the study of Lopes et al. (2020), even though the milk industry has a very large market share, there are expectations for increase in the dairy alternatives market from USD 17.3 billion to USD 29.6 billion during the forecast 2018-2023 at a Compound Annual Growth Rate (CAGR) of 11.4%. This is the biggest market share represented by the Asia-Pacific region. Besides, according to Boukid et al. (2021), the dairy alternatives market is predicted to reach USD 2.22 million at the end of the 2021-2026 forecast period at a CAGR of 7.12% in Europe. The non-dairy market is expected to reach USD 40.6 billion in the same stated period at a CAGR of 10.3% in the global market.

All food products are subject to the general rules and food marketing rules. In Europe, the Food Information Regulation EU/1169/2011, which is a major part of food law, defines the labeling rules applied to all types of food. These regulations aim for the protection of consumers and producers (Lähteenmäki-Uutela et al. 2021). Regulation (EU) No 1308/2013 of the European Parliament and Council defines the organization of the single European market and establishes a common market organization for

agricultural products (CMO). According to Annex VII of the CMO, "dairy products" are defined as the products that are derived from only "milk" which is defined as "mammary secretion obtained from one or more milking". Thus, alternatives to dairy products must have different names other than dairy associated ones (Leialohilani and de Boer 2020). Names of alternative dairy products cannot include the reserved dairy names even if they are used with “vegan” or “plant-based” words as clarifying designators based on a decision which was made by the European Court of Justice in 2017. Besides that, there are different rules for meat products. Although alternative meat products cannot be referred to as “meat”, it is allowed that they can be named with the words which describe composition or shapes of meat products such as steaks, burgers, and sausages (Lähteenmäki-Uutela et al. 2021). According to the Republic of Türkiye Ministry of Agriculture and Forestry (2020), the information “suitable for vegans/vegetarians” can be stated for food products considering the consumer sentiment.

Due to high demand many studies have been conducted related to vegan foods and new alternative products. Bedin et al. (2018) did a study with the aim of developing adequate alternative recipes to produce two traditional Italian meat products without using animal source. The review of Boukid and Gagaoua (2022) is about vegan eggs, which are a healthier alternative because of their no cholesterol content compared to eggs, and their application in other food products such as biscuits, pasta, or mayonnaise. To produce a new alternative product, which can be replaced with cow’s milk, chickpea and coconut were used and investigated in terms of nutritional composition and acceptability in the study of (Rincon, Braz Assunção Botelho, and de Alencar (2020). Lopes et al. (2020) carried out research about producing a high-protein pulse beverage, which consists of chickpea, pea, and lupin seeds, with acceptable flavor and zero-waste concept. In another study chickpea, lentil and rice flours were used to produce a lactose- and gluten-free vegan yogurt style snack using the selected species of Lactobacillus plantarum and Lactoplantibacillus brevis (Pontonio et al. 2020).

1.2. Legumes and Pulses in Vegan Diet

The edible dry seed from legumes are called pulses. Legumes and pulses such as dry pea, dry and faba beans, chickpea and lentil have lysine-rich protein (20-30%) content

(He, Meda, et al. 2021). Pulse proteins contain essential amino acids complementary to cereals (Lopes et al. 2020). Thus, they can be used as a main protein source in a plant-based diet. Environmental considerations are one of the reasons for veganism. Nitrogen is a needed source for plant growth in many crops. However, pulses, especially faba bean and chickpea, convert atmospheric dinitrogen to organic nitrogen. Therefore, the use of pulse proteins causes lower environmental impact (He, Meda, et al. 2021). Also, seed coat of legumes contains high amounts of phenolic compounds besides acting as a barrier for the cotyledon which contains relatively lower concentration of polyphenols. Polyphenols are the main antioxidants and pulses such as chickpeas, and lentils have a potential to contain high levels of antioxidant. According to literature, antioxidant activity of bioactive compounds, that are found in pulses is one of the factors, helps to understand the effects of pulses on human diet in the context of reducing the incidence of chronic diseases (B. Singh et al. 2017).

Chickpea (*Cicer arietinum* L.) is a widely consumed legume around the world and rich in minerals such as calcium, zinc, and magnesium as well as proteins (21-25%) and fibers. There is no allergenic property of chickpea which is registered officially unlike soybean (Rincon, Braz Assunção Botelho, and de Alencar 2020). It is one of the essential foods in developing countries and has a high market share which is expected to grow (He, Meda, et al. 2021). Although soybeans are very common and other pulses have lower protein content, 0.5% of the population is affected by soy allergens, so alternatives are needed like chickpea. Chickpea contains higher carbohydrates, less protein and fat in comparison to soybean. The main carbohydrates in soybean and chickpea are sucrose and starch, respectively. The use of chickpea can show some beneficial effects such as reducing risk of type-2 diabetes and blood pressure due to its high amount of resistant starch and amylose (Wang, Chelikani and Serventi 2018).

Mung bean (*Vigna radiata*), that is also known as green gram or moong bean, is a good source of protein (20-25%) and rich in iron (Dahiya et al. 2015; Ganesan and Xu 2018). Carbohydrates are the main nutrient (55-65%) in it. The primary storage proteins are albumin (25%) and globulin (60%) and the primary carbohydrate is starch. Besides that, mung bean contains tannis, trypsin inhibitors, phytic acid and some other antinutrients help to eliminate toxins. Also, mung beans involve phenolic acids, flavonoids, and other organic acids in their content. Those secondary metabolites can promote human health (Ganesan and Xu 2018).

Lentil (*Lens culinaris*) is one of the most grown pulses around the world in 2018 (Boeck, Sahin, et al. 2021). It is another gluten-free and cheap source of protein (21-31%) which contains all the essential amino acids and are rich in leucine, lysine, aspartic acid, glutamic acid, and arginine. Lentil consists of mainly starch with low glycemic index as carbohydrate (62-69%) and fibers (5-20%) and oligosaccharides besides protein content. Lentil consumption is quite popular especially in Mediterranean reagent in last years because of the potential health benefits related to decrease the risk of chronic disease such as type-2 diabetes, cardiovascular diseases, and hypertension as well as favorable nutritional composition of this legume. However, lentil proteins can cause some allergenic reactions in pediatric patients. Those proteins are generally protease resistant and heat stable. That's why it is important to consider that subject when using lentil as an ingredient. Also, obtaining a well-balanced amino acid profile can be more possible when lentil was consumed in company with the other sources of plant protein since lentil proteins have low sulfur containing amino acids and tryptophan (Romano et al. 2021). Besides that, lentil and mung bean have a better balance in terms of other amino acids than those low in sulfur containing ones and can show high antioxidant activity (Matemu, Nakamura, and Katayama 2021). Additionally, protein contents of lentils, mung beans and chickpeas' sprouts were determined by carrying out Kjeldahl and Lowry methods and the protein amounts in those pulses were lined as lentils, mung beans, and chickpeas from the highest to lowest (Rizvi et al. 2022).

There is an increasing interest in pulse supplemented new product developments. Using protein extracts of pulses, including chickpea, mung bean, lentil, pea, smooth pea, and winged pea, to form bean curd can be given as an example (H. Wu et al 2015). Besides that, development and commercialization of plant-based milk alternatives have grown around the world. When an alternative product is developed against milk, similar and satisfying composition is necessary as well as accessibility (Rincon, Braz Assunção Botelho, and de Alencar 2020). The Food and Agriculture Organization (FAO) also promotes the consumption of pulse because of their nutritional composition, benefits for soil health as well as economic accessibility (Boeck, Sahin, et al. 2021).

Plant based milk alternative term defines the water-soluble extracts of cereals, seeds, and legumes. The production processes are usually the same for all legumes. Basically, previously soaked raw materials (pulses or legumes) are processed with water

and then the extracts are filtered to obtain a liquid without pulp. If there are some other ingredients such as sugar and stabilizers, homogenization, stability, and pasteurization processes are carried out after those ingredients are added (Rincon, Braz Assunção Botelho, and de Alencar 2020).

One of the main challenges to develop a plant-based milk alternative from legumes is characteristic “beany” flavor which is derived from antinutritional compounds like isoflavones and saponins. Therefore, they can be mixed with some other compounds with the aim of solving this problem (Rincon, Braz Assunção Botelho, and de Alencar 2020). Also, this problem is related to endogenous lipoxygenases in pulses which have over 20% oil content such as soy and peanuts. Although there are expectations about less occurrence of this limiting factor in pulses that have lower oil content such as lupin and chickpea (Lopes et al. 2020), chickpea extracts have “beany” flavor, too (Rincon, Braz Assunção Botelho, and de Alencar 2020). Heat inactivation can be used to remove off flavor from the beverages. However, some undesirable results can be obtained such as lower protein solubility, highly denatured proteins, aggregation, and nutrient losses by applying this method. Besides, to suppress the “beany” flavor of soy beverages, a high temperature vapor flash treatment (at 130℃) is carried out or the beans are cooked before milling. In this way, protease inhibitors are also inactivating, and allergenic reactions can be reduced. Nevertheless, vitamin and protein denaturation are observed at the end of these processes and denatured proteins occur a residue called “okara”. As a result, production yield and nutritional potential of the beverage are decreased (Lopes et al. 2020).

1.3. Vegan Mayonnaise and Mayonnaise Based Products

Mayonnaise is a semi-solid emulsion which is composed of generally different types of vegetable oil (70-80%), egg yolk, salt, and vinegar (Hijazi et al. 2022). Food oil emulsions such as salad dressing and mayonnaise contain egg yolk, egg white or whole egg, which are natural emulsifiers for both water in oil and oil in water emulsions. The emulsifying capacity of egg is high due to its lipoproteins, phospholipids, and non-associated proteins content (He et al. 2019). In multiphase systems like mayonnaise, which is formed with emulsifying oil droplets in an aqueous phase, mentioned proteins

act as surface-active substances since they have amphiphilic properties (He et al. 2019; Raikos, Haye, and Ni 2019).

Meanwhile, according to Raikos, Haye, and Ni (2019), nowadays egg yolk has been trying to be removed from the mayonnaise formula by the food industry. Pulse proteins can be used instead of egg yolk as emulsifiers (Angelis 2022). The viscous liquid, which is obtained from cooking chickpea seeds or some other legumes in water, called as aquafaba (AQF) is commonly used as an egg replacer in vegan mayonnaise, meringue and baked goods because of its desirable emulsifying and foaming capacities. It can also be obtained from the recovered liquid of chickpea can (He et al. 2019) and AQF contains health promoting compounds such as polyphenols and high amount of protein (Lafarga et al. 2019). In fact, AQF is quite popular, but there are some challenges to be used. It is not completely explored in comparison to other egg or dairy alternatives such as plant-based proteins, hydrocolloids, and starch. Another challenge in usage of AQF is its lower protein, amino acids and vitamin content compared with milk or egg. Also, the optimum conditions cannot be provided to produce AQF due to nonstandard chickpea seeds differing from batch to batch, and different canning processes based on the brand (He, Meda, et al. 2021). Therefore, there are studies that investigate the optimization of AQF production. One of them is the study of Lafarga et al. (2019) that investigates the effects of pH, boiling conditions, the ratio of chickpea and water in weight and volume basis to improve the emulsifying and foaming properties of chickpea AQF. Tufaro and Cappa (2023) investigated the chickpea characterization and technological properties including foaming property of chickpea AQF to be used in confectionary product, specifically in meringue. In conclusion of this study, AQF, which is a recycle waste product, could have desirable technological properties, and be enhanced by addition of guar gum and lactic acid, for lowering pH, to be used in plant-based applications.

The studies related to the development of vegan mayonnaise with using egg replacers, are given in Table 1. In the study of Raikos, Hayes, and Ni (2019), it is aimed to develop a vegan mayonnaise recipe using chickpea aquafaba and to determine the effects of this recipe on physicochemical properties and texture of mayonnaise. In addition, it was stated that 70-75% oil can be replaced by the required amount of AQF to obtain a reduced fat mayonnaise in the same formula. In another study, egg-free mayonnaise recipe was trying to be developed using Arabic gum as an egg replacer with different proportions (Ali and el Said 2020). Water is used as an ingredient in some

mayonnaise recipes to solve dry ingredients in it. Recent research studies on developing vegan mayonnaise with using different egg-replacers are given in Table 1.1. below.

Table 1.1. Studies related to developing vegan mayonnaise with using different egg-replacers

Purpose	**Ingredients**	**Study**
Developing vegan mayonnaise that contains AQF from chickpea instead of egg yolk and optimization of the recipe	*Oil (80%)* *AQF (15%)* *Vinegar (4%)* *Salt (0.5%)* *Sugar (0.5%)*	Raikos, Haye, and Ni (2019)
Investigating the antimicrobial and antioxidant properties of Arabic gum. Comparison of commercial mayonnaise and alternative unpasteurized mayonnaise (partially and totally egg-free) with regards to its microbiological, chemical, physical and sensory properties.	*Corn oil (65%)* *Water (13%)* *Arabic gum* *Vinegar (10%)* *Mustard (3.25%)* *Salt (0.75%)* *Sugar (2%)*	Ali and el Said (2020)
Investigating the texture and sensory properties of egg-free mayonnaise that contains protein isolates from from chickpea, faba bean, and yellow split lentils and compare with a control mayonnaise contain a whole egg.	*Sunflower oil (70%)* *Water (17.3%)* *White wine vinegar (5.7%)* *Sugar (2.4%)* *Salt (0.8%)* *Mustard powder (0.6%)* *Xanthan gum (0.2%)* *Chickpea, faba bean, and yellow split lentils proteins (3%)*	Armaforte, Hopper, and Stevenson (2021)

(cont. on next page)

Table 1.1 (cont.)

To produce an egg-free mayonnaise with using by-product gums as egg yolk replacers	*Sunflower oil (30%)* *Water* *Lecithin (1%)* *Different types of gum*	Hijazi et al. (2022)
Obtaining AQF from chickpea with using optimized conditions to prepare an edible emulsion and foam, then compare with ones which contain egg white proteins.	*Sunflower oil (500 ml)* *AQF (150 ml)* *Lemon juice* *Salt (1 g)*	Lafarga et al. (2019)
To determine the conditions which provide optimum functions of AQF powder as an emulsifying agent in vegan mayonnaise production with standardizing production of AQF and drying process of chickpea seeds.	*Canola oil (80 %)* *Vinegar (4%)* *AQF powder (15%)* *Salt, sugar (0.5% each)*	He, Purdy, et al. (2021b)

According to the Food and Drug Administration (FDA), salad dressing is categorized as emulsified semisolid food which is made using several ingredients such as vegetable oils, acidifying agents, egg yolk-based ingredients and starchy paste. Salad dressings contain vegetable oil and egg yolk at least 30% and 4% in the weight base, respectively (FDA 2022). Vegetable oil and protein are the main ingredients of salad dressings. Vegetable oil is the primary source of fatty acids, vitamins, such as vitamin E and K, and minerals, such as calcium, iron, potassium, and some other minerals (Yin et al. 2022). The food industry works to develop some alternatives to egg since it is a common food allergen and egg yolk has a high cholesterol content. Plant-based proteins such as soybean, pea, lupin, and wheat proteins have been studied to understand their potential to be used as emulsifiers instead of egg yolk (Ma and Boye 2013). In the study of (Angelis et al. 2022), it was stated that there are many strategies that have been carried out replacing egg-yolk, reducing fat content, and enhancing the nutritional quality of salad dressings. In this context, a reduced fat vegan salad dressing formulation was developed using chickpea flour and the textural properties of the product were investigated considering the effects of chickpea flour and other ingredients. According to the results,

there was a significant effect of chickpea flour and water content on texture. Also, the safety of product is contributed with the pH value lower than 4 (Angelis et al. 2022).

1.4. Lactic Acid Bacteria (LAB) and Fermentation

LAB are included in a heterogenous bacteria group. They are Gram positive, acid tolerant, non-spore forming, non-motile, rod or cocci shaped bacteria. LAB can be found in various types of environments which are ranged from foods, such as dairy, meat, sourdough, and vegetable products, to mucosal surfaces of human body such as gastrointestinal tract, oral cavity, and vagina due to their adaptation abilities. LAB are mainly used as starter cultures for the fermentation of many types of food products in the food industry (Bintsis 2018). LAB which are included in *Lactobacillus, Leuconostoc* and *Streptococcus* genera are commonly used for fermentation. Besides other types of bacteria, fungi and yeasts can also promote fermentation (Rezac et al. 2018). For instance, *Saccharomyces* yeasts have a significant role in obtaining fermented food products (Bell et al. 2018). There are three main pathways that include LAB in the production of fermented foods and flavor development. Those pathways are glycolysis (sugar fermentation), proteolysis (protein degradation) and lipolysis (fat degradation). In fermented food products, proteolysis is a more important biochemical pathway in comparison with lipolysis for the flavor development by the contribution of LAB. Food acidification is a primary function of LAB which produces lactic acid (Bintsis 2018).

Fermentation is a well-known antient technique used for preserving foods and beverages while improves nutrition, provides better taste and food safety, and supports health properties. During the fermentation process, sugar is converted into organic acids, gases, carbon dioxide and alcohol under anaerobic conditions. This technique promotes food products with longer shelf life and safety since it removes undesirable and toxic food constituents, such as bitter tasting phenolic compounds and phytic acid, and inhibits foodborne pathogens. Moreover, it improves functionality, nutritional and organoleptic quality properties by carrying probiotic bacteria and providing the occurrence of desirable taste and enhanced nutrients (e.g., bioactive peptides and linoleic acid) (Bell et al. 2018). In the context of plant-derived source, the amount and characteristic of proteins in beans are significantly affected by fermentation. The content of cured protein in seeds can be

increased after fermentation. In grain fermentation, there are three main factors: enzyme activity, environment conditions and microbial culture. These factors are related with each other and increase the concentration of free amino acids and protein digestibility, phytic acid degradation, starch hydrolysis index and trypsin inhibitors (Maleki and Razavi 2020).

Several types of bacteria, such as *Streptococcus, Bifidobacterium*, and *Bacillus,* beside *Lactobacillus* and yeast or mold, such as *Saccharomyces, Candida,* and *Aspergillus,* are accepted as probiotics (Bell et al. 2018). Probiotics are defined as living microorganisms which confer health benefits to the host when they are ingested in sufficient amounts (Pimentel et al. 2021). LAB *Lactobacillus* and *Bifidobacterium* are the most used genera as probiotics in the food industry. Their species are mostly recognized as generally-recognized-as-safe (GRAS) (Valero-Cases et al. 2020). According to Republic of Türkiye Ministry of Agriculture and Forestry (2006), a food product can be referred as probiotic food when they contain at least 1×10^6 colony forming unit (CFU) viable probiotic microorganisms per a gram until the end of storage time. According to Republic of Türkiye Ministry of Agriculture and Forestry (2009), and Joint FAO/WHO Codex Alimentarius Commission (2011), labelled microorganism must be minimum 10^6 CFU/g and sum of microorganisms must be at least 10^7 CFU/g in fermented milk products.

The functionality and composition of the gut microbiota are altered by probiotics. They can neutralize harmful microorganisms which affect tissues of the digestive tract and regenerate out microflora and have also an ability to restore and renew those tissues. Serious disorders which are generally thought related only to psychology have a hypersensitivity to gut stimuli. Anorexia, autism, posttraumatic stress disorder are some examples of mentioned disorders (Bell et al. 2018). While probiotics show some beneficial effects associated with the immune system, gastrointestinal health, obesity, cancer, and chronical diseases, it can be simply said that they provide overall health and well-being (Bell et al. 2018; Pimentel et al. 2021).

The composition of gut microbiota can also be modulated by dietary patterns and components. These components can cause significant changes in the microbiome as well as metabolizing into microbial-derived metabolites by the microbiota. Therefore, there are many studies that research the interaction between gut microbiota and food

compounds within the context of their health effects in humans. For instance, complex carbohydrates can be fermented by gut microbiota that possesses carbohydrate enzymes, and some metabolites including short-chain fatty acids can be generated. Microbiome has an influence on the metabolisms of proteins, and lipids and the synthesis of some vitamins such as vitamin B group and vitamin K. Macro-nutrients including proteins, lipids, carbohydrates, and several micro-nutrients have considerable effects on the diversity of gut microbiota. Besides that, the composition and function of microbiota can be regulated by the effects of dietary polyphenols in a prebiotic-like manner. In this way, pathogenic developments are inhibited while the growth of beneficial bacteria is provided. In addition, dietary patterns have a considerable impact on gut microbiota. The dietary patterns that involve high amounts of sugar, saturated fat, and animal derived proteins and in contrast lower vegetables and plant derived fibers intake show negative effects on the gut microbiota. On the other hand, plant-based diets, and dietary patterns with higher consumption of vegetables have been associated with positive changes in the composition of microbiota and the production of bacterial metabolites which promote health (Ramos and Martin 2021).

1.5. Fermented Vegan Products

The development of novel food products that have gained functional properties by addition of bioactive compounds and probiotics is one of the results of searching for a healthier diet (Pimentel et al. 2021). There are studies about the use of chickpea and lentil flour in probiotic fermented milk and salad dressing to provide enrichment of the nutritional quality of the original foods (H. Wu et al 2015). Dairy products have been focused as probiotic carriers for a long time. However, non-dairy matrices have gained popularity as probiotic carries due to the increase in demands of vegan and lactose intolerant individuals (Valero-Cases et al. 2020; Pimentel et al. 2021). Fermentation of plant-based milk alternatives has become popular during the last years (Wang, Chelikani and Serventi 2018). Nevertheless, it is more challenging to maintain the viability of probiotics in a non-dairy matrix in comparison with a dairy matrix (Valero-Cases et al. 2020). *Lb. acidophilus, Lb. plantarum, Lb. rhamnosus, Lb. casei, Bifidobacterium genus*, *St, thermophilus*, *Bacillus coagulans*, and the yeast *Saccharomyces cerevisiae* are some of the probiotics used in non-dairy or vegan food products (Pimentel et al. 2021).

Soy milk is the most common plant-based beverage (Wang, Chelikani, Serventi 2018). Besides soy milk, rice and coconut milks are widely used as probiotic carriers (Rasika et al 2021). There are also studies mentioning about other legumes such as chickpea as an alternative probiotic carrier to soybean in fermented beverages (Valero-Cases et al. 2020). Wang, Chelikani, and Serventi (2018) was referring to the allergenic effect of soybean and studied on developing a fermented and unfermented chickpea beverage to be an alternative to soy milk. The fermentation was carried out with using *St. thermophilus, Lb. bulgaricus,* and *Lb. acidophilus*. As a result, the fresh chickpea beverage can be accepted as a substitute for soy milk in terms of nutritional and organoleptic quality. Besides those, the milk of mung bean, which is another pulse with high protein content, can be a useful LAB carrier. However, there is limited resource related to the proteolytic activities of LAB used in pulses (H. Wu et al. 2015).

Also, alternative yogurt products are generally produced by fermenting aqueous extracts of several legumes and oil seeds, which have similar consistency and appearance to cow's milk, but mostly manufactured from soy, almond and coconut by applying breakdown and homogenization (Grasso, Alonso-Miravalles, and O'Mahony 2020; Boeck, Zannini, et al. 2021). Plant-based yogurts, which contain similar amounts of protein as dairy yogurt, are preferred to promote individuals because of the necessity of health maintenance with adequate protein intake. There were studies investigating the other pulses or their protein isolates, such as black beans and lupin protein isolates, with the aim of using them as a base material for alternative yogurt products (Boeck, Zannini, et al. 2021).

In the study of Boeck, Zannini, et al. (2021), an alternative milk, which has the potential to contain an equal protein amount to soy and dairy products and has also a good techno functional and sensory characteristic, was tried to be produced. That's why an alternative yogurt product was developed using lentil protein isolate. After this base material was fermented by standard yogurt isolates, *Lb. bulgaricus* and *St. thermophilus*, and this fermentation caused to occur post-acidification during storage time so changing rheological properties of protein gel, fermentation was realized using lactose-negative and sucrose-fermenting *Lb. bulgaricus* and *St. thermophilus* strains. Then, a comparison between the acidification of lentil protein isolate emulsion by LAB and fermented soy-based product and cow's milk was carried out in that study.

The main challenges to produce plant based or vegan yogurt are related to textural properties and appearance. Gelling agents including natural gums, starches, pectin, and agar and their combinations are also commonly used to procure an acceptable texture in gel-type products (Grasso, Alonso-Miravalles, and O'Mahony 2020). Even in dairy-based food products, hydrocolloids can be used for providing structure and viability of probiotics as prebiotics. In the study of (Haji Ghafarloo, Jouki, and Tabari 2020), gum Arabic, a natural gum, (0.25-1%) and ginger extract were added in a yogurt drink and their effects on physicochemical properties and the viability of *Bifidobacterium bifidum* were investigated for 30 days. According to the results, while *B. bifidum* count increased with the addition of 0.5% of gum Arabic, there was no significant increase in the number with increasing amount of gum Arabic. This slightly acidic gum is commonly used as gelling agents, stabilizer, or thickening ingredients in food emulsions due to its desirable emulsifying properties (Haji Ghafarloo, Jouki, and Tabari 2020). In low-fat set yoghurt, some parameters including physico-chemical properties, bacterial counts, texture, and rheology were investigated after the addition of low-methoxyl pectin (0.05–1.0%). Higher gel strength was determined with increasing amount of pectin (Khubber et al. 2021). Besides those, phase separation is a general textural problem for vegan yogurt products. Serum separation is caused by the non-continuous weak gel formation because of the destabilized proteins. Therefore, formulations of alternative yogurt products generally involve hydrocolloids for contribution of structure formation, stabilization the particles in suspension. In this way, hydrocolloids help to imitate the characteristics of dairy-based yogurt products (Grasso, Alonso-Miravalles, and O'Mahony 2020). Recent studies for fermented and unfermented non-dairy products are summarized in Table 1.2.

Table 1.2. Studies related to fermented and unfermented non-dairy products

Aim	Probiotics	Results	References
Formulating fresh and fermented chickpea-based beverages and determining fermentability, composition and acceptability of the products	*St. thermophilus,* *Lb. bulgaricus,* *Lb. acidophilus*	• In terms of nutritional and organoleptic quality, fresh chickpea beverage can be used as a substitute for soy milk. • Fermentability was comparable to soy milk.	Wang, Chelikani, Serventi (2018)
Using lentil protein isolate to produce an alternative yogurt	*St. thermophilus* *Lb. bulgaricus*	• The acidification of using base material formed a very firm gel. • Textural and rheological properties were strongly comparable with dairy yogurt.	Boeck, Zannini, et al. (2021)
Developing novel legume beverages from pea, chickpea and lupin using different technological options to manufacture a product with high protein (at least 1.5%) and lower discharge of by-products and without "beany" flavor	_	• Although legume-based beverages have a balanced composition and similar protein content to cow's milk, there are technological issues associated with processing and preservation. • The optimization in processing techniques were performed to develop a competitive novel beverage with desirable features, that were related to rheological properties, protein content, appearance, and color, in market.	Lopes, et al. (2020)

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Table 1.2 (cont.)

Investigation of rheological, physicochemical, and sensory properties of commercial alternative yogurt products made from soybean, coconut, cashew, almond and hemp for determination of the effects of base materials to main quality attributes.	*St. thermophilus,* *Lb. bulgaricus,* *Lb. bifidus,* *Lb. acidophilus,* live vegan cultures, bacterial culture	• Soy and coconut-based yogurts and dairy-based products are similarly appreciated in terms of sensorial property. • The textural parameters of the selected yogurts were comparable to the dairy yogurt. • Soy-based yogurt products had the most similar protein content with control dairy yogurt.	Grasso, Alonso-Miravalles, O'Mahony (2020)
Developing a vegan milk product made from chickpea and coconut as an alternative to cow's milk	_	• Vanilla extract was added because of the low acceptance. • The final plant-based milk alternative was composed of 70% chickpea extract and 30% coconut extract and 0.3% vanilla extract. • The developed product can have a potential as a substitute in terms of nutritional quality (protein and calcium content), acceptance and low allergenicity.	Rincon, Braz Assunção Botelho, and de Alencar (2020)
Evaluating the fermentability of a beverage based on chickpea and coconut extracts and determining the effects of sugar contents on the bacterial growth and stability during the storage period.	*Lb. paracasei* LBC 81	• While the beverage was a viable matrix, sugar content was not a factor that guaranteed the fermentation. • Intense acidification can be a limiting factor which affects the storage time.	Mesquita et al. (2020)

1.6. Hypertension and Angiotensin-I Converting Enzyme Inhibitory Activity (ACE-I Activity)

Hypertension can be defined as high or elevated blood pressure, and it is a serious disease which causes to increase the risk of other diseases such as brain, heart, and kidney. Hypertension is diagnosed and treated in 42% of adults, besides the adult population with hypertension are not aware of their conditions (estimated 46%). Blood pressure is represented by two numbers; the first one is called systolic number and the second one is called diastolic number which presents the blood pressure in vessels when the heart beats, and the pressure in the blood vessels when the heart rest, respectively (WHO 2021). Hypertension can be classified as primary or essential and secondary types. While 95% of the cases can be classified as primary and there is no etiological cause, the secondary hypertension may be caused by pregnancy, kidney disease, Cushing's syndrome, cardiovascular problems, and side effects of drugs (Daliri, Lee and Oh 2017; Kaur et al. 2021). Besides that, there are some risk factors, such as inflammation, hypercholesterolemia, and obesity, which increase the potential prevalence of primary hypertension (Daliri, Lee and Oh 2017). Reducing the prevalence of hypertension is one of the global targets of the World Health Organization (WHO). For preventing high blood pressure, physical activities are suggested as well as a balanced diet including more fruits and vegetables, lower salt intake and fat content. Reduction in alcohol and tobacco consumption is also suggested. Besides those, reducing stress, checking, and treating high blood pressure and management of other diseases are helpful to manage this medical condition (WHO 2021).

Blood pressure is controlled by some pathways including fluid and electrolyte balance, the kinin-kallilrein, the neutral endopeptidase, the renin-angiotensin, and the endothelin-converting enzyme systems. The renin-angiotensin system (RAS) has been widely studied among the physiological mechanisms of hypertension. This system is maintained by angiotensin-I converting enzyme (ACE), renin and two proteases (Daliri, Lee and Oh 2017). ACE is a central enzyme in the RAS system and controls blood pressure. Renin enzyme acts on angiotensinogen, which is a polypeptide derived from the liver, and converts angiotensinogen (inactive form) to angiotensin I (Ang I). Then, Ang I is converted into the active hormone angiotensin II (Ang II) by the action of ACE. This

active hormone binds with receptors which are found on the vascular wall and lead blood vessels to construction. Bradykinin (hypotensive peptide), which is generated from kininogen, has hypotensive effect by the way of nitric oxide mediated vasodilation. ACE cleaves to inactive peptides including bradykinin. ACE inhibitors cause a decrease of vasoconstricting peptide by acting as a barrier and inhibits the production of Ang II. Thus, the degradation of vasodilatory peptide, bradykinin, is reduced. In this way, a reduction in blood pressure is realized (Donkor et al. 2007; Kaur, et al. 2021; Shobako 2021). The mechanism of ACE inhibition is shown in Fig. 1.1.

Since the extreme ACE activity causes hypertension, inhibiting this enzyme could be a solution for this disease (Maleki and Razavi 2020), antihypertensive medication, such as synthetic ACE inhibitors, alpha, beta, and calcium channel blockers, can be used to treat hypertension. However, using drugs have some side effects including headache, coughing, fast heart rates, etc. Thus, use of food proteins is chosen instead of synthetic drugs because of their natural origin and bio functionality. The use of food derived peptides are generally accepted as safer than drugs (Kaur et al. 2021). Different food proteins have already been used as a source of peptides that show ACE inhibitory (ACE-I) activity. Several types of fermented dairy products can be given as examples which are used for isolation of biologically active peptides (Donkor et al. 2007). Besides that, biologically active peptides and amino acids can be released by bioprocessing of legumes or pulses such as fermentation, germination, and enzymatic hydrolysis. Moreover, different types of processing including germination, fermentation, hydrostatic pressure, soaking, heat treatment and enzymatic proteolysis have a role in the generation of a remarkable number of bio-accessible peptides and phenolic compounds.

There are several methods carried out to determine ACE activity such as spectrophotometry, high-performance liquid chromatography, fluorimetry, and bioassay methods. A spectrophotometric method which was developed by Cushman and Cheung (1970), is commonly performed in food industries. The base of this method is the hydrolysis of hippuryl-histidyl-leucine (HHL) by ACE and production of hippuric acid (HA) and histidyl-leucine at the end of reaction (J. Wu, Aluko and Muir 2002). It was aimed to develop an alternative HPLC method using HHL as a substrate in the study of J. Wu, Aluko and Muir (2002). Also, ACE activity determination assay, which is based on the hydrolysis of another substrate furanacryloyl–prolyl-glycylglycine (FA-PGG), was

suggested by Holmquist et al. (1979). Then, this method with a different substrate was tried to be developed by some other studies. In those studies, antihypertensive drugs such as captopril was used as a positive control or a standard (Vermeirssen, van Camp, and Verstraete 2002, Hou et al. 2003). The methods using mentioned two substrates, HHL and FA-PGG, were compared in the studies of Shalaby, Zakora, and Otte (2006) and Henda et al. (2013).

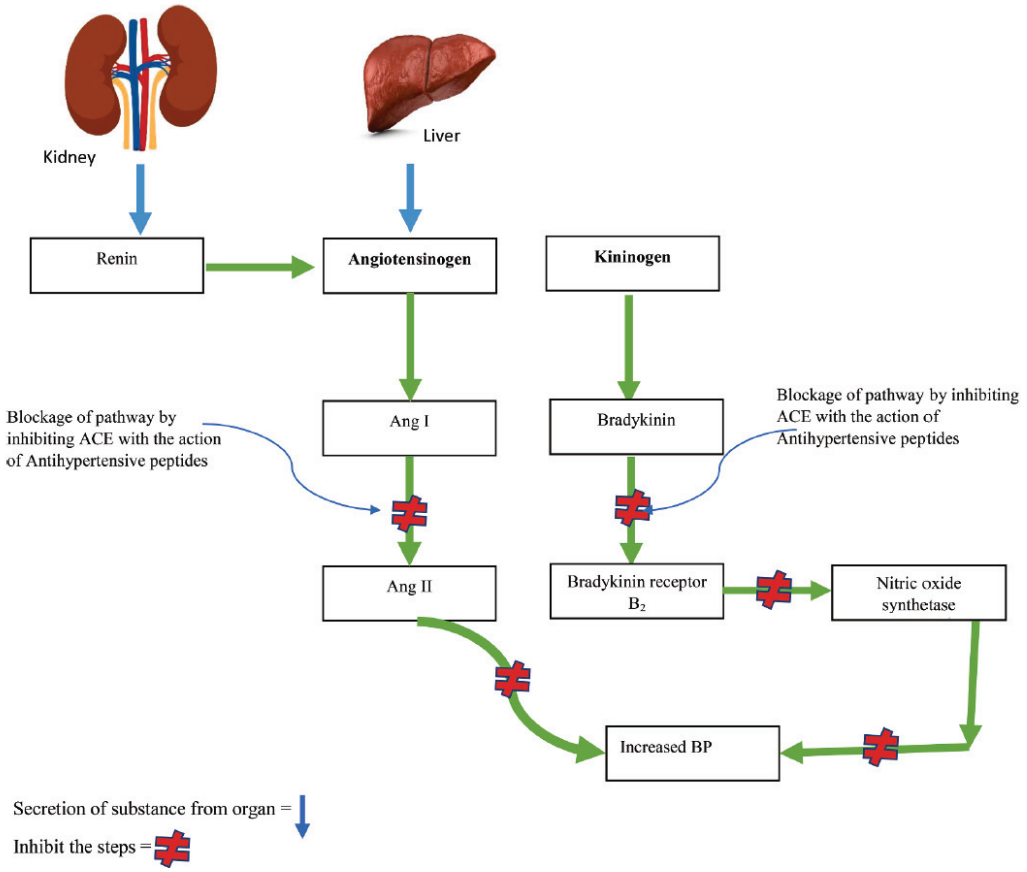

Figure 1.1. ACE inhibition mechanism (Source: Kaur, et al. 2021)

As mentioned before, gut microbiota has a role in the overall well-being and human health. In addition to that, there are several studies about the impact of microbiota on physiological homeostasis including blood pressure. The imbalances in the presence, reciprocal abundance, and localization of the bacteria species in the gastrointestinal tract are related to hypertension based on several studies. Thus, the attempt of using proper and suitable diet to fix disturbances in the microbiota for controlling high blood pressure

has been raised. The functionality of probiotics related to their impact on blood pressure have been discovered in last years. Use of probiotic bacteria and fermented food products seems to have a potential for controlling hypertension. Bioactive peptides, such as ACE-I peptides, are released with the fermentation of foods by probiotics. If the probiotics, such as *Lb. helveticus*, *Lb. rhamnosus*, *Lb. reuteri*, and *Bifidobacterium*, are found in desirable amounts, the generation of nitric oxide, a vasodilator, can be promoted in microphages so that, vasodilation is enhanced, and high blood pressure can be reduced. When the polyamines are reduced in the vasculature, blood pressure is decreased and some probiotic bacteria, such as *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lb. plantarum*, *Lb. casei*, have the ability of reducing polyamine levels in tissues. Also, antioxidant abilities of probiotics may have a role in reducing blood pressure. Superoxide dismutase is produced by some of the probiotics, besides some of them have metal chelating abilities. Therefore, it can be said that the regulation of vascular relaxation and contraction is one of the properties of bacteria. Probiotics can increase the solubility and absorption of calcium ions. The absorption of dietary calcium inhibits the uptake of extracellular calcium and suppress renin; thus, blood pressure is lowered in patients with hypertension (Daliri, Lee and Oh 2017).

Inoculation of LAB in legumes shows beneficial health effects. For instance, the bio accessibility of polyphenols and proteins, which have health promoting properties including antihypertensive effect, in lentil can be increased by fermentation. Processing methods, kind of pulses (the protein content), sequence and weight of released peptides, the proteolysis are some of the factors that affect the health benefits of bioactive peptides associated with the inhibitory activity of ACE. The potential ACE-I activity can be increased or decreased by proteolysis within the gastrointestinal enzymes which remove amino acid residues. Although lipase or glucosidase digestion can circumstantially provide the generation of bioactive peptides which have antihypertensive property, ACE I activities of peptides depend mainly on the sequence and composition of amino acids. Therefore, gastrointestinal stability and bioactive peptides stability against the degradation of gastrointestinal enzymes have significant effects on the potential ACE inhibition (Maleki and Razavi 2020). In product base, if proteolysis is low, ACE inhibition is also showing a low degree (Garbowska, Pluta, and Berthold-Pluta 2020), and low proteolytic activity of LAB is one of the limitations of using those bacteria for

legumes fermentation since the sufficient bioactive peptides from legumes proteins cannot be easily released in that type of situation (Maleki and Razavi 2020).

Although fermentation and germination are two inexpensive bioprocessing methods that can be carried out for the enrichment of ACE-I activity and reduction of anti-nutritional factors, sometimes they may cause the reduction of ACE inhibition and high inhibition activity is found in the seed's crude extracts only. The quality and quantity of protein content of legumes is one of the factors that affects weight and kind of peptides sequencing and the proteolysis; and also, is a factor for determining the amount of releasing peptides during mentioned processes. If all pulses amino acids, non-allergenic protein found in pea, low amount of antinutritional factors which affect the digestibility of nutrients in chickpea and cowpea, and intestinal microflora modulating ability of chickpea are considered and a good balance is provided, pulses can have a good potential to be used instead of hypertension drugs (Maleki and Razavi 2020). Some studies associated with the ACE-I activity in fermented and unfermented plant-based products and sources are in Table 1.3.

In the study of Barbana and Boye (2010), ACE-I activity of protein hydrolysates which are obtained from yellow pea and two kinds of chickpea with using alcalase/flavourzyme and papain (gastrointestinal enzymes) was determined in vitro. As a result, proteins of these pulses contain ACE inhibitory peptides and using enzyme type for hydrolysis is a factor that affects the ACE-I activity (Barbana and Boye 2010). In another study, the fermentation conditions for producing a product which has a potential to be a source of ACE-I peptides that is obtained from fermented pea seed hydrolysates was investigated. According to results, *Lb. plantarum* was used for fermentation and this process may enhance the releasing ACE-I peptides during in vitro digestion. It was suggested that food products such as chips or pasta can be produced by using fermented seeds which has beneficial effects on health (Jakubczyk et al. 2013).

Table 1.3. Studies related to ACE inhibitory (ACE-I) activity in fermented and unfermented plant-based source and products

Topic	Legumes	Microorganisms	Results	References
▪ Soymilk as a substrate ▪ Growth and acid development at different pH ▪ Proteolytic and ACE inhibitory activities of probiotics	Soybean	*Lb. delbruekii ssp. bulgaricus,* *St. thermophilus,* *Lb. acidophilus*, *B. lactis*, *Lb. paracasei*	• Survival of probiotics - not strain dependent. • Appreciable ACE inhibitory and proteolytic activity	Donkor et al. (2005)
▪ ACE inhibitory activity ▪ Different hydrolysates obtained from chickpea and yellow pea. ▪ Using in vitro gastrointestinal simulation, alcalase/flavourzyme, and papain.	Chickpea (kabuli and desi) & yellow pea (Golden)	_	• Containing bioactive ACE inhibitory peptides • The type of enzyme affects the ACE-I activity	Barabana and Boye (2010)
▪ Fermentation conditions of pea seeds ▪ Potential ACE-I activity after fermentation and in vitro digestion	Pea seeds	*Lb. plantarum* 299v	• There was no ACE-I activity after fermentation. • Release of antihypertensive peptides potentially during digestion	Jakubczyk et al. (2013)

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Table 1.3 (cont.)

▪ Solid and liquid state fermentation of lentil ▪ Antihypertensive and antioxidant properties	Lentil	Natural fermentation (microorganisms present on the seeds) *Lb. plantarum* *Bacillus subtilis*	• Lentil fermentation is a suitable process for obtaining water soluble extracts having potential antihypertensive compounds and antioxidant properties.	Torino et al. (2013)
▪ Mung bean milk ▪ The fermentation process and proteolysis effect	Mung bean	*Lb. plantarum* B1-6	• Higher ACE inhibitory activity at the end of fermentation • Good carrier for LAB	H. Wu et al. (2015)
▪ The functional attributes of fermented soy milks	Soybean	*Lb. plantarum* C6, *Lb. rhamnosus* C8, *Lb. rhamnosus* C25, *Lb. rhamnosus* C28, *Lb. rhamnosus* C34, (isolated from cheese) *Lb. helveticus* NCDC 288 (reference strain)	• Some bio-functional components during soymilk fermentation • Health beneficial soy foods or bio-therapeutics with selected strains	B. P. Singh, Bhushan and Vij (2020)

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Table 1.3 (cont.)

▪ Germinated brown rice. ▪ Fermented yogurt-like product	Brown rice	LAB strains	• Fermentation of brown rice formulations • Improvement of the bioactive compounds and ACE-inhibitory activity	Cáceres et al. (2019)

In the study of Donkor et al. (2005), it was aimed to understand soymilk's suitability as a substrate for acid development and growth by some probiotic strains at different pH values and investigate the ACE inhibitory and proteolytic activities of these microorganisms. For fermentation of soy milk specific species of *Lb. bulgaricus, St. thermophilus*, and other probiotic organisms (*Lb. acidophilus, Bifidobacterium lactis and Lb. paracasei*) were used. Consequently, soy yogurt, that was produced at the end of fermentation by using yogurt starter cultures and mentioned probiotic strains, showed higher ACE-I activity in vitro in comparison with the control sample which was produced by using only starter cultures. This situation could be caused because of higher proteolytic activity of probiotics. In soy yogurt, different pH values did not show any effect on the viability of probiotics and survival of probiotics was also not strain dependent. The purpose of H. Wu et al. (2015) was to prepare mung bean milk that is supplemented by sucrose, then investigate and optimize the proteolysis effect of *Lb. plantarum* B1-6 and its fermentation capacity of mung bean milk. As a result, ACE-I activity was significantly higher after the fermentation process than before.

According to several studies, applying diets based on plant sources and rich in vegetables would be much healthier. Veganism is a lifestyle and a diet that has gained popularity in last years. So, today vegan food products are in high demand. Also, the beneficial properties of fermentation and use of probiotics have been known for years. Antihypertensive effect against high blood pressure is one of the beneficial properties of health promoting bacteria. Therefore, the purpose of this thesis is to develop fermented vegan food formulations showing antihypertensive effect with ACE-I activity.

CHAPTER 2

MATERIAL AND METHODS

2.1. Material

Chickpea, mung bean, and yellow split lentil, which are used for preparation of chickpea pre-culture (CP pre-culture) and plant-based milk alternative (PBMA), besides salt, sugar, sunflower seed and olive oils, lemon, and apple vinegar, which are the ingredients of vegan mayonnaise, were purchased from local markets. Strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria were obtained from Izmir Institute of Technology Food Engineering Department Molecular Food Microbiology Laboratory culture collection.

2.2. Methods

2.2.1. Preparation of The Products

Preparation methods for chickpea pre-culture (CP pre-culture), fermented plant-based milk alternatives (F.PBMA), vegan mayonnaise, and salad dressings (SDs) were determined in this section.

2.2.1.1. Chickpea Pre-culture

First, CP pre-culture was prepared for adapting microorganisms to a plant-based media according to the undergraduate theses of Tığ (2020) and Karaman (2020) with some modifications. Chickpeas were soaked for 12 h using distilled water at room temperature. After soaking water was drained, different ratios of chickpea (1:4, 1:6 and 1:12 (w/v)) and chickpea flour (1:4 and 1:10 (w/v)) were tested to obtain chickpea milk

alternative (CP milk alternative) by using distilled water. They were blended using a hand blender and filtrated through two-layer cheese clothes. Different pasteurization parameter such as 75-80℃ for 15 min, 65-70℃ for 15 min, 65-70℃ for 30 min, and finally 72℃ for 20 min were tested and carried out in an autoclave (Hirayama). 1.5% glucose (AppliCam), and 1% yeast extract (Merck) were added into the pasteurized CP milk alternative in aseptic conditions. 3 different combinations of *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* strains, which were previously tested in skim milk in the study of a graduation projects of Tığ and Karaman (2021), were investigated within this chickpea media (CP media). First, bacteria were cultivated in De Man, Rogosa and Sharpe (MRS) broth (Merck) and M17 broth (Biolife) in the ratio of 1% from stock cultures stored at -80℃. After an incubation for 24h at 42℃, an activation (1%) was carried out at the same incubation parameters. Then, the CP media was fermented by yogurt isolates (2% each) at 42℃ for 5-6 h. Preparation steps of CP pre-culture were shown in Fig. 2.1.

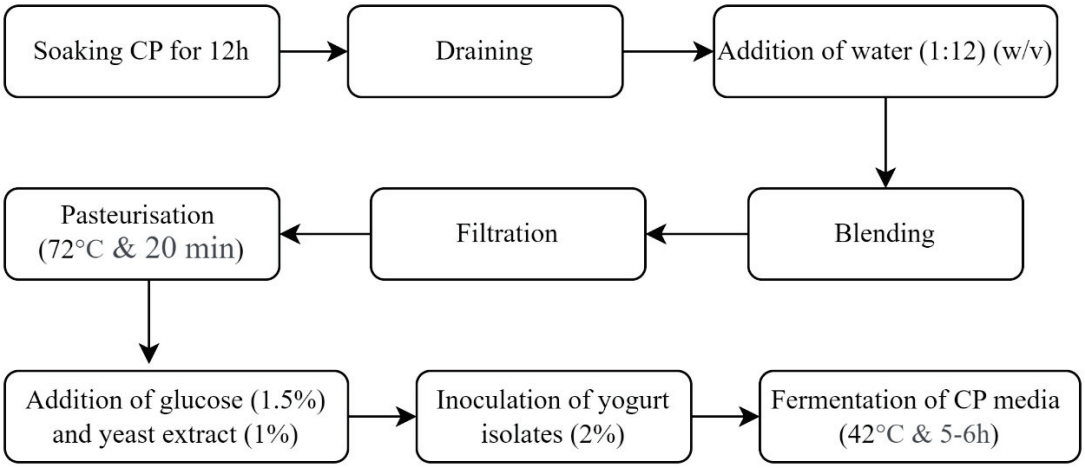

Figure 2.1. Flow chart of CP pre-culture preparation

2.2.1.2. Preparation of Fermented Plant-Based Milk Alternative

To prepare a mixed PBMA, pulses (chickpea, yellow split lentil, mung bean) were soaked for 12 h at room temperature. After draining the excess water, a different ratio of distilled water was added for each soaked pulse. While tested ratios of water for chickpea and mung bean were 1:6 and 1:8 (w/v), for yellow split lentil 1:2, 1:4 and 1:6 (w/v) ratios

were tried. The ratios of lentil and mung bean was decided to be tested according to the studies of Torino et al. (2013) and H. Wu et al. (2015). The milk alternative manufacturing process used in the preparation of chickpea pre-culture was carried out in order to obtain milk alternative from these pulses. Pasteurization was carried out at 65-70℃ for 15 min for the first and second trials, 65-70℃ for 30 min for third, fourth and fifth trials, and 72℃ for 20 min for the sixth and seven trials.

In the first trial, 3 types of milk alternatives were pasteurized separately at 65-70℃ for 15 min and inoculated by pre-culture (2%), which was prepared by using the ratio of 1:12 (w/v) chickpea/distilled water and selected combination of bacteria strains, to understand the fermentation capacity of bacteria in mung bean (1:8), and yellow split lentil (1:6) milk. Besides that, mung bean and yellow split lentil were mixed (1:1) (v/v) [MB: YSL] and fermented by pre-culture (2%). Fermentation was carried out at 42℃ for 6-7 h.

In the second trial, after the MB: YSL milk (1:1) (v/v) was filtered through two-layer cheese cloths, it was homogenized at 15,000 rpm for 5 min and then pasteurized at the same temperature in the first trial. Non-homogenized and homogenized milks were inoculated with using pre-culture (2%) and directly MRS and M17 broths (2%). Also, apple pectin (Fluka) (0.25%) (w/v) was added as an emulsifier to prevent phase separation. Fermentation was continued at 42℃ and observed for 22 h and 8 h for the milks which were fermented by pre-culture and broths to reach a pH value near 4.6, respectively.

In the third trial, corn starch (from a local market) (0.5%) (w/v), gum Arabic (Fluka) (0.1%) (w/v), and agar-agar (AppliCam) (0.25%) (w/v), were added to the homogenized and pasteurized (with uploaded parameters) MB: YSL milk as emulsifiers, separately. The milks were inoculated by pre-culture (2%) and fermented at 42℃ for 22 h.

In the fourth trial, mung bean (1:8) and yellow split lentil (1:6) (w/v) were mixed with chickpea milk (1:8) (1:1:2) (v/v) [MPBMA] and homogenized, then pasteurized. After that, 1% of gum Arabic (w/v) was added into them. Inoculation from the pre-culture was carried out in two different ratios, 2% and 3%. Fermentation parameters were the same as in the first trial.

In the fifth trial, the MPBMA, which was obtained in the previous trial, was inoculated with pre-culture (3%) and 1% glucose (AppliCam) and sucrose (Sigma) were added to provide the fermentation process. In the second hour of the process, a strain of *Lb. bulgaricus* (2%), which has potentially good proteolytic activity and was activated earlier in MRS broths, was incorporated into the fermentation. In this trial fermentation continued at the same temperature as before for 5-6 h.

In the sixth trial, 25% chickpea (1:8), 35% mung bean (1:8), and 40% yellow split lentil (1:6) (w/v) [PBMA] were soaked together at room temperature for 12 h. They were mixed with total volume of distillated water based on the mentioned ratios in the same beaker at the same time unlike before, with the help of a hand blender. Then, pulse slurry was filtered through two-layer cheese cloths and homogenized using previously stated parameters. Pasteurization parameters were updated to 72℃ for 20 min both CP milk alternative and PBMA. Then, PBMA was inoculated by pre-culture (3%) [F. PBMA1]. It was allowed to be fermented without motion for 5 h.

In the seventh and final trial, PBMA, which was comprised of 3 different pulses at the stated ratios in the trial six, was inoculated by three different *Lb. bulgaricus* strains (2%) separately at the second hour of fermentation that was started by pre-culture (3%) inoculation [F. PBMA2, F. PBMA3, and F. PBMA4], besides the F. PBMA1. Fermentation was carried out at 42℃ and lasted for 5±0.5 h. Preparation steps of F. PBMAs were shown in Fig. 2.2.

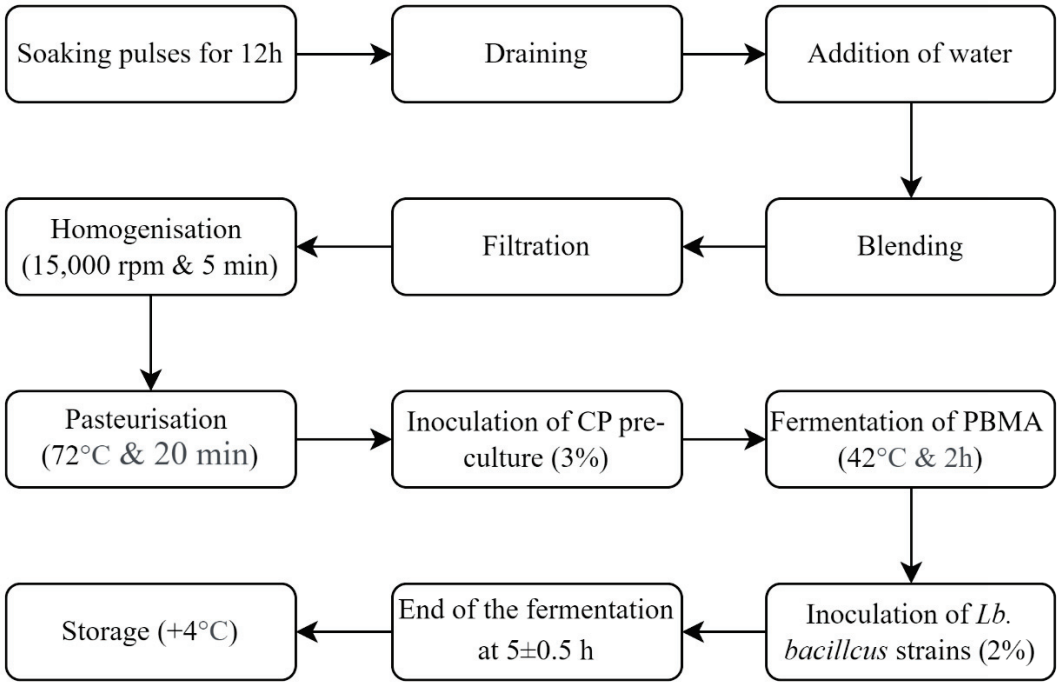

Figure 2.2. Flow chart of F. PBMA preparation

2.2.1.3. Mayonnaise Preparation

For preparing a vegan mayonnaise, aquafaba, as an egg-replacer, was prepared and tested from chickpea and water in the ratios of 1:1.5 and 1:1.7 (w/v) by using an autoclave at 115℃ for 30 min based on the studies of Lafarga et al. (2019) and He et al. (2019) with modifications. Two mayonnaise formulations were made and tested based on the studies of Lafarga et al. (2019) and Raikos, Hayes, and Ni (2019) with some modifications. While ingredients of the first tested formula were oil (75%), aquafaba (20%), vinegar (4%), sugar (0.5%), salt (NaCl) (0.5%) on weight basis, and oil (75%), aquafaba (24.8%) (adjusted pH to 3.5 using lemon juice), and salt (0.2%) were used for the second mayonnaise formula. Sunflower seed oil and olive oil were used in the formulations. In total, 8 different vegan mayonnaise were obtained. All ingredients, except oil, were put in a beaker and stirred by hand. Then, oil was added and homogenized at 14,000 rpm for 2 min using a homogenizer (IKA T25 digital Ultra-Turax). After all types of obtained vegan mayonnaises' first appearance and textural properties were observed manually, selected ones were pasteurized at 72℃ for 20 min.

2.2.1.4. Salad Dressing Preparation

First, selected types of mayonnaise made by using sunflower seed oil, were mixed with F. PBMA1 in the ratios of 1:1 to obtain vegan salad dressings. A further selection between the remaining mayonnaises was made. After that, two of them, that were prepared by using aquafaba (1:1.7 (w/v)) in the two different formulations and were mixed with F. PBMA1 (2:1 (v/v)), they all were subjected to tests. Finally, 3 types of salad dressings were obtained by mixing a mayonnaise made with using aquafaba (1:1.7 (w/v)) in the first formulation and F. PBMA 1, F. PBMA2 and F. PBMA3 in the ratio of 2:1 (v/v) under aseptic conditions. The products were stored at 4℃. Preparation steps of AQF, mayonnaise and salad dressings were shown in Fig. 2.3.

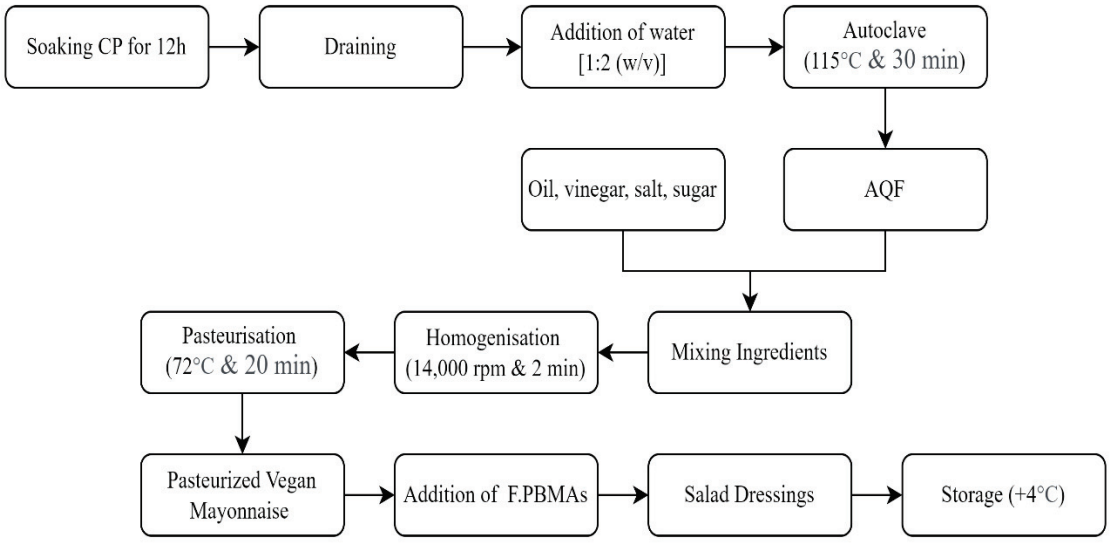

Figure 2.3. Flow chart of AQF, vegan mayonnaise and salad dressings preparation

2.2.2. Microbiological Analyses

Bacterial counting was carried out using both MRS agar and M17 agar for enumeration of bacilli and cocci, respectively for all three types of fermented plant-based milk alternative and salad dressings. Besides those, Plate Count Agar (PCA), Potato Dextrose Agar (PDA), Violet Red Bile Agar (VRBA) were used for detecting total aerobic bacteria count, yeast and mold, and coliform detection, respectively with using

spread plate method. Total bacteria count, yeast and mold detection were also applied for base materials which are mayonnaise, and plant-based milk alternative. Microbial counting was carried out for all samples each week of the shelf life in 2 parallels for 2 replicates.

2.2.3. pH Determination

Determination of the pH values of mayonnaise and salad dressings was carried out by a digital pH meter (HI 2211, HANNA Instruments, US) using 2 ml of samples at room temperature. The measurements were made each week of the shelf life for 2 replicates.

2.2.4. Determination of Brix Value

Brix values of the samples were measured by using a digital refractometer (Isolab, Germany) at room temperature biweekly for 2 replicates.

2.2.5. Determination of Titratable Acidity (TA)

The titratable acidity of samples was determined based on the standard method which was stated in Nielsen (2017a) with some modifications. 2 g of sample was diluted to 20 ml and phenylphthalein indicator was added, nearly 3 drops, into the diluted sample and stirred. After 50 ml of 0.1 N NaOH (Applicam) was prepared and poured into a burette, it dropped into the samples until their color turned pink. The measurements were carried out biweekly during the shelf life for 2 replicates. The titratable acidity of mayonnaise and salad dressing was calculated by the following equation (Nielsen 2017a; Tly and Sadler 2017):

$$\%Acid = \frac{V\ (ml) \times N\ of\ titrant\ \left(\frac{mEq}{mL}\right) \times Eq.wt\ of\ acid(\frac{mg}{mEq})}{Sample\ volume\ in\ mL\ (g) \times 1000\ (mg/g)} \times 100 \quad (1)$$

where V is volume of titrant, N is normality of titrant, Eq. wt. is equivalent weight.

The equivalent weight of acetic acid was calculated by the following formula (Tly and Sadler 2017):

$$Equivalent\ weight = \frac{Molecular\ weight}{The\ number\ of\ equivalents} \quad (2)$$

Since the molecular weight of acetic acid is 60.06 and equivalents per mole is 1, equivalent weight of acetic acid are given as 60.05 in Tly and Sadler (2017). The results were expressed based on the percentage of acetic acid in the sample.

2.2.6. Protein Analysis

Protein content of mayonnaise and salad dressings were determined using Kjeldahl method based on AOAC (1996) with some modifications. It was carried out in IZTECH Biotechnology and Bioengineering Application and Research Centre. In this procedure, 1 g of samples, 20 ml of sulfuric acid, antifoaming agent and one catalyzer tablet were put into Kjeldahl tubes and heated at 450℃ for 5 h. After degradation, the organic nitrogen content was converted to ammonium sulfate. In 4 min distillation part, 70 ml of NaOH (40% (w/v)) and 100 ml of distilled H_2O were added for neutralization and forming ammonia, after that, 70 ml of boric acid (3%) was added and ionize ammonia to attach with HCl (0.1M) in titration process with using Vapodest 50s distillation and titration unit (Gerhardt GmbH & Co., Germany). Using nitrogen-to-protein conversion factor was 6.25 that is also used as general factor. The measurements were performed in three parallels for mayonnaise and salad dressing samples with one replicate, and the results were expressed as percentage of protein g^{-1}. Total nitrogen and total protein contents were calculated by using the following equations:

$$\%N = \frac{1.4007 \times T \times F(Consumption\ of\ HCl - Blank\ value)[ml]}{Content\ [g]} \quad (3)$$

$$\%\ Protein = \%N \times Protein\ factor \quad (4)$$

where T is titer (molarity of HCl), %N is total nitrogen in the samples.

2.2.7. Emulsion Stability Index (ESI) and Emulsifying Activity Index (EAI)

The emulsion stability index (ESI) and the emulsifying activity index (EAI) were determined using the method of Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak (2022) with some modifications. 50 μl of the emulsions, mayonnaise, and salad dressings, was taken and diluted to 8 mL of 0.1% sodium dodecyl sulphate (SDS), then vortexed for 10s with using a vortex. The absorbance of the diluted samples was measured in plastic cuvettes, which have 1 cm path length, with using a UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) at 500 nm. The ESI and EAI were calculated using the following formulas:

$$EAI(m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times \phi \times 10000} \quad (5)$$

$$ESI(min) = \frac{A_0}{A_0 - A_{10}} \times t \quad (6)$$

where A_0 and A_{10} are the absorbance values of diluted samples at the initial time and 10 min respectively, c is the protein concentration (g/ml), N is the dilution factor (160), φ is the oil volume fraction (0.75), ϕ is an optical path (1 cm), and t is the time interval. The average protein concentration of each sample which was measured in the protein analysis was used for the EAI calculation of the same sample. The measurements were carried out in 2 parallels and were reported as the mean ± standard deviation (n =2).

2.2.8. Moisture and Ash Content

The moisture content of 4 g of mayonnaise and salad dressings were measured by drying under reduced pressure using vacuum oven at 20mm Hg at 20℃ for 12 hours. After that, ash content of these samples was measured using muffle oven at 575 ± 25℃ for 3 h. Those determinations were performed in the initial and final weeks of the shelf life for 1 replicate. The following formulas were used for calculations (Marshall 2010; Nielsen 2017b):

$$\%\ Moisture\ content\ \left(\frac{wt}{wt}\right) = \frac{(\text{wt of wet sample} + \text{pan}) - (\text{wt of dry sample} + \text{pan})}{(\text{wt of wet sample} + \text{pan}) - (\text{wt of pan})} \times 100 \quad (7)$$

$$\%\ Ash\ (dry\ basis) = \frac{wt\ after\ ashing - tare\ wt\ of\ crucible}{original\ sample\ wt \times (dry\ sample\ wt - tared\ crucible\ wt)} \times 100 \quad (8)$$

2.2.9. Mineral Analysis

Mineral contents of plant-based milk alternative, mayonnaise, and salad dressings were measured based on the study of Sathivel et al. (2005) with some modifications by using Inductively coupled plasma - optical emission spectrometry (ICP-OES) (5110, Agilent Technologies, US) in IZTECH Environmental Development Application and Research Center. After deformation process of samples was carried out using 10 ml of nitric acid and 2 ml of hydrogen peroxide in a closed vessel at high temperature and pressure, measurement was carried out in the 3rd week of shelf life for 1 replicate. Calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphate (P), and zinc (Zn) elements were searched in the samples and the results were given as mg kg^{-1}.

2.2.10. Color Analysis

Before analysis all samples were mixed with the help of a vortex. 5 ml of each sample were poured into glass peri dishes (10 cm diameter). Then, the color of the samples was measured by using a chroma meter (Konica Minolta CR-400, Japan). The CIE L*a*b system color parameters were expressed in terms of L* (lightness), a* (redness) and b* (yellowness) values (Wlodarczyk, Zienkiewicz and Szydlowska-Czerniak 2022). The measurements were carried out in 3 parallels for 2 replicates in the first and final weeks of the shelf life. The whiteness index (WI) is calculated by the following equation (Boeck, Zannini, et al. 2021):

$$WI = 100 - \sqrt{((100 - L^*)^2 + a^{*2} + b^{*2})} \quad (9)$$

2.2.11. Total Phenolic Content Analysis

Mayonnaise and salad dressing samples were prepared according to Romeo et al. (2021) with some modifications. 0.5 g of sample was diluted with 4.5 ml of ethanol and centrifuged at 9,000 rpm for 5 min at 10℃. Then diluted samples were filtered using a 0.2 mm pore size nylon filter. After diluted samples were filtered, they were diluted further with ethanol to the ratio of 1:500 and 1:100 (w/v) for mayonnaise and salad dressings, respectively. The phenolic content of samples was determined using the method in the study of Cemeroğlu (2013) with some modifications.

2 ml of Folin-Ciocalteu's phenol reagent (Fluka) (10% v/v) was added into 500 μl of samples and allowed to mix for 5 min. Then, 1 ml of sodium carbonate (Merck) solution (7.5% w/v) was added into them and kept in the dark at room temperature for 30 min. After 30 min the absorbance of the solution was measured at 765 nm with UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) against ethanol. Also, ethanol was added instead of the samples as a control. All measurements were made in 3 parallels during the shelf life biweekly. The results were denoted as mg gallic acid L^{-1} of

sample. Gallic acid standard curve, which was drawn in the study of Atik (2022), was used to determine the results.

2.2.12. Antioxidant Activity (DPPH, ABTS) Assay

For antioxidant analysis, sample preparation method mentioned in the 2.2.11. Total Phenolic Content Analysis was applied.

ABTS stock solution was prepared according to the study of Re et al. (1999). ABTS (Rache) (7 mM) was dissolved in distilled water with 2.45 mM potassium peroxodisulfate (Sigma) to produce ABTS radical cation. Before the use of solution, it stored for 12-16 h at room temperature in the dark. The absorbance of the solution was adjusted to be read within the range of 0.70 ($\pm$ 0.2) with dissolving into ethanol. The assay was carried out by using the studies of Re et al. (1999) & Romeo et al. (2021) with some modifications. 50 μl aliquot of the sample and 2950 μl of ABTS solution were mixed and the absorbance was spectrophotometrically measured at 734 nm against ethanol after1 min by using a UV-visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Ethanol was added instead of the sample as a control.

DPPH solution was prepared using the values in the study of de Bruno et al. (2021) with a modification; 6×10^{-5} M DPPH (Aldrich) was dissolved in ethanol. According to the Romeo et al. (2021), 2950 μl of DPPH solution and 50 μl of the sample were mixed in a cuvette and kept in the dark for 70 min. Then, the absorbance was determined spectrophotometrically at 515 nm against ethanol. The percentage of inhibition was calculated by the following formula:

$$\%Inhibition = \frac{A_{t0} - A_{t70}}{A_{t0}} \times 100 \quad (10)$$

where A_{t0} is the initial absorbance value of DPPH solution and A_{t70} is the measured absorbance value after 70 min. The measurements were biweekly made in 3 parallels.

Trolox (Aldrich) standards (from 1.5 to 50 μl) was dissolved in ethanol and the absorbance values were read using the same methodology. The results were expressed as μmol Trolox ml^{-1} of sample for ABTS and % inhibition for DPPH.

2.2.13. OPA Analysis

The proteolytic activity of the Lb. bulgaricus strains, present in the samples, was subjected to preselection test using skim milk agar (10% skim milk) within the scope of the TÜBİTAK Project (1190112). It was concluded that they may potentially show high proteolytic activity by observing the zone that was formed around the bacterial colony.

The proteolytic activity of the samples was measured by carrying out the o-phthaldialdehyde (OPA) test from Pescuma et al. (2010) with some modifications. Eight ml of TCA (Merck) (0.75%) and 4 ml of mayonnaise and salad dressing samples were allowed to wait at room temp for 30 min. After that, samples were centrifuged at 5000 rpm for 10 min with using centrifuge (Universal 320R, Andreas Hettich GmbH & Co. KG, Germany) and their supernatants were stored at -20℃ until assay was carried out. 1.25 ml of SDS (AppliChem) (20% (w/v)), 12.5 ml of sodium tetraborate (Fluka), (100 mmol/L), 20 mg of OPA (Sigma) dissolved in 500 μl of ethanol, 50 μl of beta-mercaptoethanol (Merck) and 10.700 μl dH_2O were added for reach 25 ml of OPA solution which was prepared on daily basis.

Then, 200 μl of OPA solution and 10 μl of test sample, which was diluted with distilled H_2O 1:2 (v/v), were put into wells of 96-well plates (Cellstar) in five parallels. After an incubation at 37℃ for 5 min in Varioskan Flash (Thermo Electron Corporation, U.S.), the results were spectrophotometrically determined at 340 nm. The measurements of only OPA solution placed in each well were used as controls for each measurement. The measurements were made in 5 parallel with 2 replicates.

L-leucine (Sigma) was used for drawing the standard curve (0.05-0.6 mg/ml dH_2O). The measurements were carried out with two replicates and results were expressed as mg Leu ml^{-1} sample.

2.2.14. ACE Inhibition (ACE-I) Activity Assay

To prepare samples for the ACE-I analysis, the method of (Pihlanto, Virtanen, and Korhonen 2010) was carried out with some modifications. After the samples were centrifuged at 10,000 rpm for 20 min at 4℃, their supernatants were stored at -20℃ until the analysis. Before the analysis was carried out, pH values of samples were arranged to 8.3 using 2.5 M NaOH.

The assay was performed based on the method of (J. Wu, Aluko and Muir 2002) with some modifications. The substrate, HHL and ACE were dissolved in borate buffer (100mM), containing 0.3M NaCl (Merck) and 1 M HCl for the adjustment of pH value to 8.3. The assay was performed in 1.5 ml Eppendorf tubes; the total reaction volume was 350 µl consisting of 250 µl HHL solution (2mM), 50 µl ACE (Sigma) solution (30mU/ml), and 50 µl sample. The substrate solution and sample were mixed, and maintained at 37℃ for 10 min meanwhile, ACE was incubated at the same parameters. After 10 min, ACE was added into the substrate solution and sample mixture then, the reaction was carried out for 30 min at 37℃. The reaction mixture was gently shaking by hand every 10 min. After 30 min, the reaction was stopped by the addition of 100 µl HCl (1 M) and the reaction mixture filtered through a 0.20 mm pore size nylon filter. The assay buffer was used instead of inhibitors (samples) and included into the HHL in the control sample. In the blank, the assay buffer was mixed with HHL, and sample mixture instead of the enzyme.

The HPLC analysis was carried out with Diode-Array Detector (DAD). Symmetry C_{18} column (3.0×150 mm, 5µm, Walters) was used. HHL and HA were detected at 228 nm. Mobile phases were 0.05% Trifluoroacetic acid (TFA) (Sigma) in water and 0.05% TFA in acetonitrile.

Isocratic elution was carried out at the constant flow rate 0.5 ml min^{-1}. Injection volume was 50 µl and the analysis temperature was 30℃. HA standard samples were prepared on a daily basis and used to draw a standard curve and control the results during the experiment. Captopril was used as a positive control (Hou, et al 2003). The assay was carried out in duplicates with two replications. The percentage of ACE inhibition was calculated by the following formula of (Shalaby, Zakora and Otte 2006; Gonzalez-Gonzalez, Tuohy and Jauregi 2011):

$$ACE - I\ \% = ([HA_{control} - HA_{sample}]/HA_{control}) \times 100 \quad (11)$$

where $HA_{control}$ and HA_{sample} represent the conversion of peak areas into hippuric acid in terms of μM based on the standard curve in the absence and presence of samples, respectively.

2.2.15. Shelf-Life Analyses

The salad dressings were stored at 4℃ because of the probiotic content. Yeast and mold, coliform, total viable count, enumeration of lactic acid bacteria and pH value determination were carried out on 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} and 35^{th} days (each week) of the storage time in the concept of shelf-life analyses.

Determination of brix value, titratable acidity, antioxidant capacity, total phenolic content, emulsion stability and emulsifying activity indices, ACE-I activity and OPA assays were carried out in 1^{st}, 3^{rd}, and 5^{th} weeks of storage. While color, moisture and ash content analyses were done in the 1^{st} and 5^{th} weeks, protein, mineral analyses were carried out in the 3^{rd} week of storage.

Also, total viable count, cultivation of coliform, yeast and mold were done for mayonnaise and salad dressing in 2^{nd} and 3^{rd} months.

2.2.16. Statistical Analyses

Averages and standard deviations were calculated by Excel. Statistical significance was determined by applying one-way analysis of variance (ANOVA), and the comparison of data was made by Tukey test using Minitab 17 Statistical Software. (α=0.05)

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Preparation of The Products

The results of preparation steps for chickpea pre-culture, fermented plant-based milk alternatives, vegan mayonnaise and salad dressing formulations are given and also discussed in this section.

3.1.1. Preparation of Chickpea Pre-culture

When CP pre-culture was prepared, chickpeas were soaked in water for 12 h. Approximately 100 g chickpea absorbed 120 ml of distilled water (dH_2O). First, the ratio of CP: water (1:4) and (1:6) (w/v) and CP flour: water (1:4) and (1:10) were prepared to obtain alternative chickpea milk and autoclaved at 75-80℃ for 15 min. After the pasteurization process, thickening was high in all CP milk alternatives, and CP flour: water (1:4) could not be used because of that reason. The other three ratios were prepared one more time and filtration of slurry was performed more slightly, CP pulp in the cheese clothes did not squeeze with the aim of less starch transfer to the milk (Fig. 3.1). Weight of CP pulp and volume of milk, which were obtained in the first and second trial, were stated in Table 3.1.

Table 3.1. CP milk alternative obtaining trials for pre-culture with using 25 g chickpea

CP milk alternatives		Pulp (g)	Milk alternative (ml)
CP: water (1:4) (w/v)	1st trial	30	70
	2nd trial	64.5	72
CP: water (1:6) (w/v)	1st trial	19-20	114
	2nd trial	57.5	135

(a) (b)

Figure 3.1. CP pulp after filtration first trial (a); second trial (b) of chickpea milk alternative preparation

After glucose (1.5%) and yeast extract (1%) were added into each CP milk alternative (Fig. 3.2), 15 ml of CP media were transferred in autoclave bottles within two parallels and in aseptic conditions. A strain of *Lb. bulgaricus* (bty 73), which has potentially high proteolytic activity, was cultivated (2%) into the CP milk alternatives. While one of the parallels was allowed to be fermented for 24h, another one was used to control pH values which is shown in Table 3.2. Phase separation was observed in CP flour: water (1:10), CP: water (1:6), and CP: water (1:4) milk alternatives from more to less. The most precipitate formation was observed in CP: water (1:4) milk alternative. Thus, it was decided to try the cultivation of yogurt starter cultures using CP: water (1:6) milk alternative.

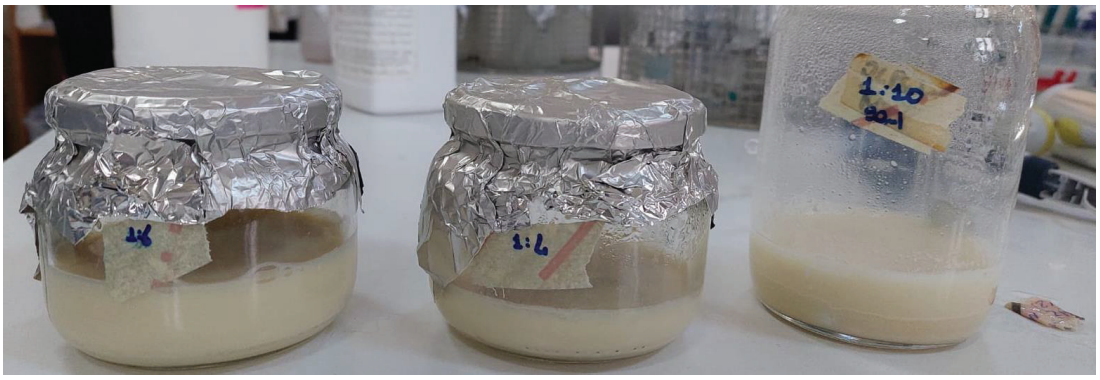

Figure 3.2. CP milk alternative with different concentrations after pasteurization

Table 3.2. pH values of fermented CP media by *Lb. bulgaricus* (bty 73) (McFarland value: 7.9) for 24 hours.

CP milk alternative	Milk alternative	4.5h	5.5h	24h	
				Controls	Main
1:4	6.52	5.61	5.44	3.85	3.52
1:6	6.52	5.63	5.26	3.56	3.48
1:10 (with CP flour)	6.34	5.74	5.53	3.51	3.39

After CP: water (1:6) media was prepared, divided into equal portions in autoclave bottles and 3 different combinations of starter cultures (2% each) were inoculated, *Lb. bulgaricus* strains were coded as bty 73, 8b and 69 and *St. thermophilus* strains were coded as cty 41 and 44 (Fig. 3.3). McFarland values of used bacteria were stated in Appendix A. The pH values were controlled as stated in Table 3.3.

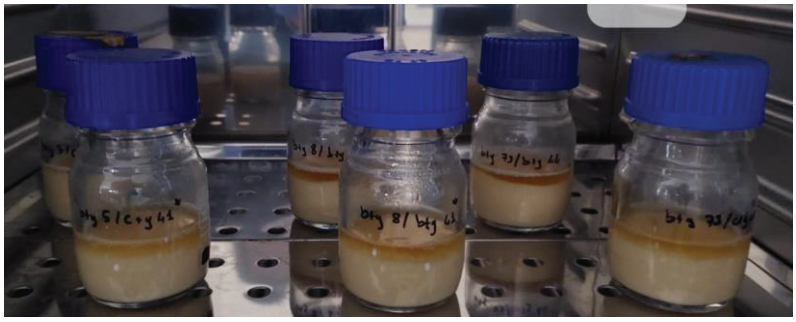

Figure 3.3. CP: water (1:6) media inoculated by different combinations of yogurt isolates

Table 3.3. pH values of CP: water (1:6) pre-culture inoculated with different combinations of yogurt isolates during fermentation

	0 h	5 h	6 h	24 h
Bty5 & Cty41	6.23	5.71	5.85	3.46
Bty8 & Cty41	6.12	5.93	5.82	3.29
Bty73 & Cty44	6.15	5.31	5.11	3.26
CP milk	6.63			6.60
CP media	6.50			6.60

The precipitation in milk was higher than before probably due to the preparation of milk and pre-culture with larger amounts or pasteurization parameters. In addition to that, phase separation was high because pH values could not be lower than pH 5.0 in 6 hours and fermentation time was prolonged. Therefore, while pasteurization parameters were updated as 65-70℃ for 15 min, CP milk was prepared in the concentration of 1:12 (CP: water) (w/v) to prevent excessive precipitation. pH values of this testing were stated in Table 3.4. During the fermentation of CP: water (1:12) media, bottles were gently stirred at 0, 2.5 and 5th hours in order to prevent the phase separation. However, phase separation was nearly two times higher than before and there was no yogurt like texture. Nevertheless, CP pre-culture inoculated with bty 73 & cty44 bacteria combination had a desirable pH of 4.6 at the end of 5h.

Table 3.4. pH values of CP: water (1:12) pre-culture inoculated with different combinations of yogurt isolates during fermentation

	0 h	**5 h**	**6 h**	**7.5 h**	**24 h**	**After storage (+4℃)**
Bty5 & Cty41	6.15	5.71	5.60	5.34	3.58	3.45
Bty8 & Cty41	6.14	5.69	5.59	5.29	3.46	3.32
Bty73 & Cty44	5.95	4.55	-	-	-	4.38
CP milk alternative	6.61					
CP media	6.48					

3.1.2. Preparation of Fermented Plant-Based Milk Alternative

After it was decided that the use of CP: water (1:12) pre-culture inoculated by bty73 & cty44 bacteria combination, main plant-based milk alternative was tried to be obtained using yellow split lentil (YSL) and mung bean (MB) in the first place. The legume: water ratios and their milk obtaining data were given in Table 3.5.

Table 3.5. Plant-based milk alternative obtaining trials with using 20 g YSL and MB

PBMA	Absorbed water (ml)	Pulp (g)	Milk alternative (ml)
YSL: water (1:2)	25.5	29.7	39
YSL: water (1:4)	27	26.8	84
YSL: water (1:6)	29	23.8	130
MB: water (1:6)	24	29.8	119
MB: water (1:8)	23	27.5	153

After the pasteurization at 65-70℃ for 15 min, the precipitation amount in plant-based milk alternatives were YSL: water (1:2) > YSL: water (1:4) > YSL: water (1:6) > MB: water (1:6) > MB: water (1:8) for the same amount of milk (20 ml) (Fig. 3.4). Therefore, YSL: water (1:6) and MB: water (1:8) were chosen to be fermented. Also, they were mixed (1:1) (v/v) [MB: YSL] in aseptic conditions and 20 ml of each milk type were fermented by pre-culture (2%).

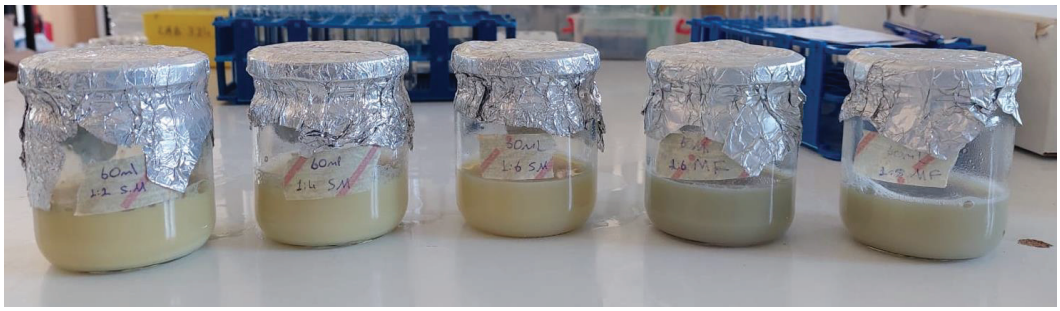

Figure 3.4. Plant-based milk alternatives after pasteurization

pH values of plant-based milk alternatives and their fermented forms were given in Table 3.6. Inoculation (2%) was performed from the CP pre-culture cultivated by bty73 & cty 44 after 24h of storage at +4℃. Fermentation was stopped at 6 h, near the pH value 4.6, and fermented milks were stored at +4℃. After 24h, their textures and odors were checked. MB: YSL was the most acceptable one.

Table 3.6. pH values of pre-culture, initial and fermented plant-based milk alternatives (20 ml) during fermentation

	Milk alternative	0 h	5 h	6 h
MB (1:8)	6.56	6.50	4.78	4.78
YSL (1:6)	6.63	6.45	5.23	4.62
MB: YSL	6.58	6.47	5.01	4.72
Pre-culture		4.38		

Plant-based milk alternatives were prepared once more with larger volume and fermentation was repeated with 100 ml of milks at the same parameters (at 42℃) to examine the physical properties and bacterial count (Fig. 3.5). pH values of this testing and its pre-culture were stated in Table 3.7 and Appendix B.1, respectively.

(a) (b)

Figure 3.5. Fermented plant-based milk alternatives after 7h (a); after 16 h of storage at +4℃ (b)

Table 3.7. pH values of pre-culture, initial and fermented plant-based milk alternatives (100 ml) during fermentation and after 16h storage at +4℃

	Milk alternative	0 h	4 h	6 h	7 h	After storage (+4℃)
MB (1:8)	6.57	6.32	5.78	5.06	4.98	5.12
YSL (1:6)	6.67	6.54	6.08	4.81	4.64	4.64

(cont. on next page)

Table 3.7 (cont.)

MB: YSL	6.66	6.53	5.84	4.91	4.76	4.77
Pre-culture		4.33				

Bacterial count was performed for different plant-based media in order to observe the growth of bacterial strains and the obtained values were shown in Table 3.8. There were 10^7 bacteria in fermented vegan milk samples. Phase separation occurred in all of them because of the proteolysis; longer fermentation time was observed, and the phase separation increased after 16 h of storage at +4°C.

Table 3.8. The bacterial count for *Lb. bulgaricus* and *St. thermophilus* strains, CP pre-culture and fermented milk alternatives

	10^{-8}	**10^{-7}**	**10^{-6}**
Bty 73	1	24	192
Cty 44	2	13	142
CP pre-culture	2	12	121
MB (1:8)	-	13	110
YSL (1:6)	3	22	133
MB: YSL	1	14	180

Results present the mean value of two parallel.

As the second trial to obtain fermented plant-based milk alternatives, homogenization at 15,000 rpm for 5 min was performed before pasteurization to prevent phase separation problem. Homogenized and non-homogenized MB: YSL were inoculated by pre-culture (2%) and directly *Lb. bulgaricus* and *St. thermophilus* which were allowed to grow in MRS and M17 broths (2% each). Also, the use of emulsifier was considered, and 0.25% apple pectin was added into the filtered MB: YSL before the homogenization to prevent phase separation. Table 3.9. shows the pH values of pre-culture, initial and fermented different types of milk during fermentation in the 2nd trial.

Table 3.9. pH values of pre-culture, initial and fermented different types of milk alternative during fermentation in the 2nd trial

	Milk alternative	0 h	4 h	6 h	8 h	22 h
Non-homogenized	6.55	6.46	6.17	6.0	5.48	5.16
Homogenized	6.66	6.43	6.14	5.98	5.35	5.01
Homogenized & Pectin	5.97	5.91	5.57	5.47	5.17	4.62
Non-homogenized (From broths)	6.59	6.13	5.83	5.51	5.39	-
Homogenized (From broths)	6.57	6.10	5.78	5.52	5.42	-
Homogenized & Pectin (From broths)	5.92	5.41	5.04	5.08	5.14	-
Pre-culture		4.41				

McFarland value of cty 44 is lower than normal as it is stated in Appendix A. pH value of CP pre-culture was closer to 4.6 in longer than the predicted time, CP media fermentation was stopped at 8th hour at the pH 4.75 as it is given in Appendix B.2. bty 73 and cty 44 were cultivated one more time to be used in the inoculation from directly from broths into the plant-based milk alternatives. Homogenization affected the pH and caused a color change which could be more acceptable.

The color of MB: YSL also became more like cow's milk with the addition of pectin as it is stated in Fig. 3.6 (a). After 22 h fermentation of MB: YSL (inoculated by CP pre-culture), there was far too much phase separation in all testing samples. While phase separation was lower in the milk samples, which were inoculated by broths, pectin (0.25%) successfully prevented the phase separation. However, this sample was liquid and there was almost no coagulation unlike the others (Fig. 3.6 (b)).

(a) (b)

Figure 3.6. Homogenized [MB: YSL] milk with pectin addition and non-homogenized [MB: YSL] milk alternative after pasteurization (a); fermented [MB: YSL] milk alternative by yogurt isolates from MRS and M17 broths (b)

Also, CP milk alternative, that was prepared for pre-culture, was cultivated in PCA to check the total viable cell count. After excessive unwanted bacterial growth was observed, pasteurization parameters were uploaded for both CP and MB: YSL as 65-70℃ for 30 min.

For the third trial, corn starch (0.5%), gum Arabic (0.1%) and agar-agar (0.25%) were separately used as emulsifiers in MB: YSL which was pasteurized at 65-70℃ for 30 min. Also, CP milk was pasteurized at the same renovated parameters. pH values of CP pre-culture, which was used for inoculation (2%), were stated in Appendix B.3 and data of pH control of MB: YSL versus time was stated in Table 3.10.

Table 3.10. pH values of pre-culture, initial and fermented different types of milk alternatives during fermentation in the 3rd trial

Milk alternative	0h	4h	5h	22h
MB: YSL	6.52	6.43	5.83	5.80	4.64
MB: YSL - starch (0.5%)	6.57	6.46	5.92	5.80	4.66
MB: YSL - Agar-agar (0.25%)	6.55	6.46	5.85	5.82	4.58
MB: YSL - Gum Arabic (0.1%)	6.38	6.23	5.65	5.62	4.61
Pre-culture		4.31			

Besides all fermented MB: YSL were thickened, there was an off odor and phase separation in all samples due to the long fermentation time. The structure of samples was shown in Fig. 3.7. The lowest pH at 5th hour was measured for MB: YSL - Gum Arabic (0.1%) and the firmest structure belonged to that sample. After the cold storage (+4℃), there were some textural changes. While MB: YSL - Agar-agar (0.25%) caused a high level of gelation and nearly had a solid structure, the structure of MB: YSL- Gum Arabic (0.1%) was clearly fragmented. However, the off odor continued.

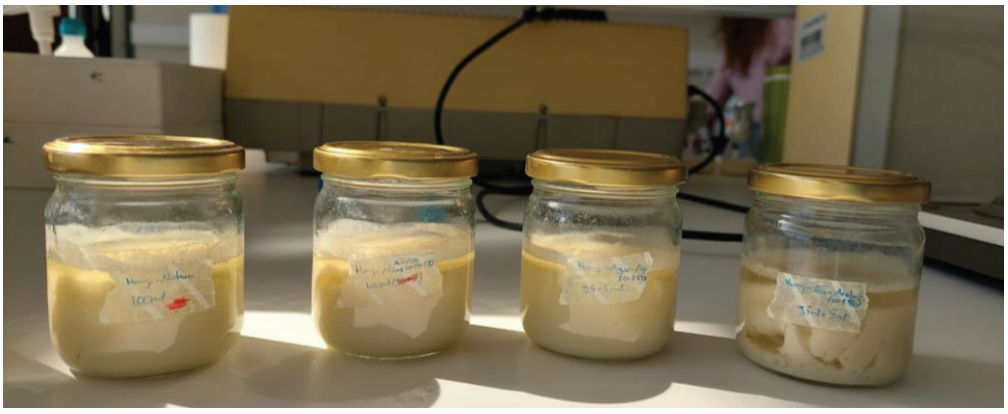

Figure 3.7. Fermented MB: YSL milks in the concept of 3rd trial (homogenized MB: YSL inoculated by CP pre-culture, with addition of starch, agar-agar, and gum Arabic from left to right)

As the 4th trial, chickpea milk was decided to be included into MB: YSL milk in the ratio of 1:1 for providing the milk with more starch content and increasing similarity of the media in favor of bacterial growth after CP pre-culture. For this purpose, previously prepared CP milk in 1:6 and 1:8 (CP: water) ratios, which were pasteurized at 65-70℃ for 15 min, and fermented by CP pre-culture (2%), which was prepared with CP milk alternative pasteurized at the same parameters, were used for consideration. The structures of fermented CP milks were shown in Fig. 3.8 and their pH values were given in Table 3.11.

Table 3.11. pH values of pre-culture, initial and fermented CP milk alternative in the concept of 4th trial

	Milk alternative	0 h	24 h
CP (1:6)	6.58	6.35	4.16
CP (1:8)	6.62	6.48	4.12
Pre-culture		4.48	

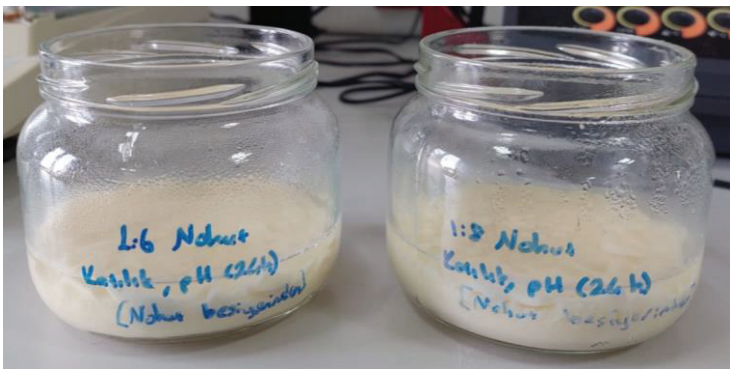

Figure 3.8. Fermented CP (1:6) and CP (1:8) milk for 24 h

Fermented CP milk (1:8) was preferred to be used for low precipitation both in milk and fermented forms, lower pH, and more acceptable odor. In brief this mixed plant-based milk alternative [MPBMA] consisted of 25% MB (1:8), 25% YSL (1:6) and 50% CP (1:8) milk alternative. In this time, 1% of gum Arabic was added into the testing samples due to the supporting ability about pH in the previous trial. Also, different ratios of inoculation from CP pre-culture were tested (2% and 3%). The pH values of CP pre-culture and MPBMA samples were shown in Appendix B.4. and Table 3.12 for 7 h of fermentation, respectively.

Table 3.12. pH values of pre-culture, initial and fermented MPBMA by CP pre-culture (2% and 3%) in the 4th trial

	Milk alternative	0h	4 h	5 h	7h
MPBMA- pre-culture (2%)	6.59	6.45	6.05	5.88	5.71

(cont. on next page)

Table 3.12 (cont.)

MPBMA- pre-culture (3%)		6.42	5.96	5.75	5.59
MPBMA- Gum Arabic (1%)- pre-culture (2%)	6.26	6.04	5.82	5.62	5.51
MPBMA- Gum Arabic (1%) - pre-culture (3%)		6.08	5.58	5.46	5.38
Pre-culture		4.48			

There was an unpredictable situation related to the pH values, which could not be closer to near 4.6. There was off-flavor and phase separation especially in MPBMA- Gum Arabic (1%) inoculated with 2% pre-culture as it is shown in Fig. 3.9.

Figure 3.9. Fermented MPBMA in the concept of 4th trial inoculated by CP pre-culture (2%) and with addition of gum Arabic (1%), and inoculated by CP pre-culture (3%) and with addition of gum Arabic (1%) (from left to right)

In the fifth trial, to obtain a shorter fermentation time, glucose, and sucrose were added into MPBMA that was inoculated with pre-culture (3%). A strain of *Lb. bulgaricus* (bty 71) was incorporated into the fermentation. pH values of CP pre-culture were stated in Appendix B.5, and fermented MPBMA were given in Table 3.13, respectively.

Fermentation of MPBMA samples provided by glucose addition was stopped at 5th h because the pH values were desirable at that point. pH values of all samples were close to each other at the end of fermentation without a huge time difference. However, there was an excessive phase separation and no texture formation in all samples as is

shown in Fig. 3.10. *Lb. bulgaricus* strain, coded as bty 71, was one of the bacteria that had potentially high proteolytic activity. Increasing proteolysis may be the cause for phase separation. Also, pH values were similar to each other even the addition of bty 71. This could be caused by limited nutritional sources in the MPBMA. Therefore, the pH could not be lowered effectively because of the competition among the mentioned 3 bacteria. Also, CP milk alternative and MPBMA were cultivated in PCA, and aerobic growth was observed.

Table 3.13. pH values of pre-culture, initial and fermented MPBMA by CP pre-culture (3%) and bty 71 (2%) in the 5th trial

	Milk alternative	**0h**	**2.5h**	**4h**	**5h**	**5.5h**
MPBMA – pre-culture (3%)	6.58	6.36	-	5.56	5.28	4.86
MPBMA – pre-culture (3%) – glucose (1%)	6.53	6.35	-	4.99	4.37	-
MPBMA – pre-culture (3%) – sucrose (1%)	6.54	6.42	-	5.32	5.01	4.86
MPBMA – pre-culture (3%) – bty71 (2%)	6.58	6.37	6.15/5.81	5.40	5.20	4.96
MPBMA – pre-culture (3%) – glucose (1%) – bty71 (2%)	6.53	6.38	5.79/5.25	4.92	4.57	-
MPBMA – pre-culture (3%) – sucrose (1%) – bty71 (2%)	6.54	6.38	5.99/5.32	5.28	4.95	4.79
Pre-culture		4.42				
Bty 71			4.32			

Figure 3.10. Fermented MPBMA milks in the concept of 5th trial

In the sixth trial, because of the excessive bacterial growth in PCA and suspicion of the insufficient pasteurization, parameters were changed from 65-70℃ for 30 min to 72℃ for 20 min. PBMA consisted of 25% of chickpea (1:8), 35% of mung bean (1:8), and 40% of yellow split lentil (1:6) (w/v) was prepared and inoculated by 3% pre-culture [F. PBMA1]. pH values of pre-culture, initial and fermented PBMA by pre-culture (3%) in the 6th trial were given in Table 3.14 and pH values of CP pre-culture that was used for inoculation were stated in Appendix B.6.

Table 3.14. pH values of pre-culture, initial and fermented PBMA by pre-culture (3%) in the 6th trial

	Milk alternative	**0 h**	**5 h**	**After storage (+4℃)**
PBMA – pre-culture (3%) [F. PBMA1]	6.57	6.54	4.86	4.76
Pre-culture		4.61		

The texture of F. PBMA1 was smooth, homogenized, and viscose. There was no phase separation, off odor, and undesirable color. This preparation method was tested once more. CP pre-culture, which reached pH of 4.64 at the end of fermentation lasted for 5.5 h and pH of 4.47 after storage, was used. In this time, fermentation of PBMA, which was inoculated by pre-culture (3%), lasted for 7 h. pH values of this testing can be seen from Table 3.15. There was a yogurt-like texture, and it turned into a viscous liquid when it was stirred as shown in Fig. 3.11.

Table 3.15. pH values of pre-culture, initial and fermented PBMA by CP pre-culture (3%) in the 6th trial as repetition

	Milk alternative	0h	5h	6.5h	7h	After storage (+4°C)
PBMA – pre-culture (3%) [F. PBMA1]	6.55	6.40	5.40	5.25	5.07	4.97
Pre-culture		4.64				

Figure 3.11. F. PBMA1 in the concept of 6th trial

In the seventh trial, PBMA was inoculated by pre-culture (3%) and three different strains of *Lb. bulgaricus* (2%) were included into the half fermented PBMAs, separately. pH values of F. PBMA1, 2, 3 and 4 were given in Table 3.16 while pH values of CP pre-culture were stated in Appendix B.7. The fermentation stopped at 4.5 h. There was no phase separation in F. PBMA1. Besides that, phase separation was slightly considerable in F. PBMA2, and 3 but, more considerable in F. PBMA4. There was firm texture which turned viscous liquid when stirred. The LAB count was carried out for those samples and CP pre-culture (Appendix C.1). There were approximately 10^8 bacteria in pre-culture and all other samples. Bty 71 was eliminated from the addition as a LAB which shows potentially high proteolytic activity because of the possible competition between this bacteria and yogurt isolates based on pH values and counting results in Appendix C.1. Also, it caused slightly off-odor and more phase separation than others.

Table 3.16. pH values of pre-culture, initial and fermented PBMA by CP pre-culture (3%) and bty 8b, bty 69 and bty 71 (2% each) in the 7th trial

	Milk Alternative	0h	2h	4.5h	After storage (+4°C)
PBMA	6.65				
PBMA – pre-culture -YB (3%) [F. PBMA1]		6.38	5.90	4.87	4.84
PBMA – pre-culture -YB (3%) – bty8b [F. PBMA2]		6.38	5.05	4.60	
PBMA – pre-culture -YB (3%) – bty69 [F. PBMA3]		6.38	4.88	4.56	
PBMA – pre-culture -YB (3%) – bty71 [F. PBMA4]		6.38	4.86	4.92	
Pre-culture		4.49			
Bty 8b			4.15		
Bty 69			4.28		
Bty 71			4.41		

3.1.3. Preparation of Mayonnaise and Salad Dressing

First aquafaba was prepared from chickpea in the ratios of 1:1.5 and 1:1.7 (CP: water) (w/v). Then mayonnaise samples were prepared based on formulation 1 and 2 mentioned in part 2.2.1.3. Mayonnaise samples which were prepared using olive oil and sunflower seed oil were shown in Fig. 3.12.

As a preselection, the appearance of samples was considered, and it was decided to use sunflower seed oil in the formulations. The samples, which were stated at lower right part of Fig. 3.12, were the samples prepared using AQF (1:1.7) and olive oil in the first and second formulations. The appearance and texture of those samples were very different than others because they could not be properly homogenized. Therefore, they were criticized based on their colors.

Figure 3.12. Mayonnaise samples that were prepared with using sunflower seed oil (upper part); and olive oil (lower part)

F. PBMA1, that was prepared in the first testing of the 6th trial, was added into the unpasteurized mayonnaise samples [UPM] prepared using sunflower seed oil in the ratio of 1:1 (UPM: F. PBMA1), to understand the potential of salad dressings with a desired consistency. pH values of this testing were given in Table 3.17. Another selection was performed based on the texture of mixture and productivity of AQF preparation. The texture of samples could be aligned as UPM-formulation 2-AQF (1:1.5)> UPM-formulation 2-AQF (1:1.7)> UPM-formulation 1-AQF (1:1.5)> UPM-formulation 1-AQF (1:1.7) from more viscous to the less based on observation. Table 3.18 showed that prepared AQF with different ratios had almost the same pH but, AQF (1:1.7) was more efficient than other one based on the production volumes. After that, UPM samples from both formulations 1, and 2 and AQF (1:1.7) were cultivated in PCA to check the total viability. While the result of AQF (1:1.7) was clear, there was too much growth for UPMs. Bacterial growth in PCA for formulation 1, prepared with vinegar, was lower than the one for formulation 2, prepared using lemon juice. After those results, pasteurization was performed at 72℃ for 20 min.

Table 3.17. pH values of testing of potential salad dressing using F. PBMA1 and UPM

	Samples	**After mixing**
F. PBMA1	4.76	

(cont. on next page)

Table 3.17 (cont.)

UPM- formulation 1 AQF (1:1.5) (vinegar)	4.06	4.42
UPM- formulation 1 AQF (1:1.7) (vinegar)	4.10	4.50
UPM- formulation 2 AQF (1:1.5) (lemon)	3.42	4.07
UPM- formulation 2 AQF (1:1.7) (lemon)	3.50	4.13

Table 3.18. Results of AQF production with different ratios

	AQF (ml)	**pH**
AQF [1:1.5] [70g :105ml] (CP: water)	38	6.27
AQF [1:1.7] [70g :119ml] (CP: water)	60	6.24

The pasteurization process caused a slight decrease in pH and there was neither bacterial growth in PCA nor yeast and mold growth in PDA for both formulations. After that process, F. PBMA1, that was prepared in the second testing of the 6th trial, was added into the mayonnaise samples (2:1) (mayonnaise: F. PBMA1) and pH values were stated in Table 3.19.

Table 3.19. pH values of pasteurized mayonnaise samples, F. PBMA1 and their mixture (2:1)

Samples	**pH**
F. PBMA1	4.97
Formulation 1 (unpasteurized)	4.07
Formulation 1 (pasteurized)	4.01
Formulation 1 (past.) + F. PBMA1	4.35
Formulation 2 (unpasteurized)	3.50
Formulation 2 (pasteurized)	3.43
Formulation 2 (past.) + F. PBMA1	4.02

After being sure of the textural properties by observation, F. PBMA1 which was prepared in the 7th trial was added into two mayonnaise formulations and pH control

(Table 3.20) and LAB count were carried out for salad dressings for day 1 and 5 (Appendix C.2).

Table 3.20. pH values of salad dressings (SD) and their ingredients

Samples	pH
AQF	6.12
AQF (with lemon juice)	3.48
Lemon juice	2.25
Vinegar	3.10
F. PBMA1	4.84
Formulation 1 (pasteurized)	3.96
SD with formulation 1	4.24
Formulation 2 (pasteurized)	3.29
S. D. with formulation 2	3.93

pH of SD was checked in 1 week and there was a slight decrease (approximately 0.01). The color of the samples was also checked, SD with formulation 1 was white and SD with formulation 2 was in cream tones as they were in the beginning. Phase separation was observed at the bottom of samples, phase separation in SD with formulation 2 was more than formulation 1. However, there was no phase separation in mayonnaise samples. Besides that, SD with formulation 1 seemed to provide the LAB in it more than formulation 2. Because of that, it was decided to continue with SD with formulation 1, other word SD prepared using vinegar.

In addition to F. PBMA1, other fermented PBMAs that include *Lb. bulgaricus strains,* bty 8b and bty 69 (F. PBMA2 and F. PBMA3) were added into the mayonnaise prepared by using vinegar [SD 1, SD 2, and SD 3]. McFarland, pH, and optical density (OD) values of LAB in the fermentations were reported in Appendix C.3. Alternative milk preparation results including absorbed water, pulp (g), and obtained alternative milk (ml) were checked to compare the productivity of obtaining process for two replications (Table 3.21). Results were similar, so it can be said that obtained CP milk alternative and PBMA had similar nutritional values based on legumes.

Table 3.21. Data of milk obtaining processes

	Chickpea milk alternative	PBMA
Absorbed water	13.8 ml	62.8 ml
	13 ml	63.7 ml
Pulp	16.1 g	82 g
	18.8 g	89.5 g
Milk	145 ml (from 144 ml)	380 ml (from 388 ml)
	139 ml (from 144 ml)	370 ml (from 388 ml)

3.2. Microbiological Analysis

Pasteurization parameters were arranged based primarily on total aerobic bacteria, and yeast and mold growth. There was no unwanted aerobic bacterial or yeast and mold growth in mayonnaise and PBMA after pasteurization at 72℃ for 20 min. Coliform detection was also performed besides the mentioned counting methods for salad dressings. According to the Republic of Türkiye Ministry of Agriculture and Forestry (2011), coliform bacteria are allowed to be present in 2 samples among 5 with the range of 10^1-10^2 CFU/ml in mayonnaise and mayonnaise-based salad dressings. The results of PDA and VRBA count were clear for SDs during the shelf life. Beside those, using LAB for the fermentation could be also grown in PCA (Appendix C.4). The shelf-life of the SDs was decided to be 4-5 weeks and LAB counting was performed based on this time interval. Additionally, mayonnaise and SDs were cultivated into PCA, PDA, and VRBA in 2, and 3 months. While aerobic bacteria count was similar for SDs, there was no detection of coliform, yeast, and mold at the end of 3 months. LAB count was carried out for using bacteria, *St. thermophilus* and 3 types of *Lb. bulgaricus*, for all three types of SD during the shelf life, and additionally for CP pre-culture and F. PBMAs at the preparation time. McFarland values were checked for bacteria before the inoculations to basically ensure the similarity of the growth in broths. Bty 73, bty 8b, bty69 and cty 44 were 10^7-10^8 CFU/ml bacteria (Appendix C.5). The growth of them was consistent in CP pre-culture and F. PBMAs (Appendix C.6). Based on that, PBMA was effectively used as a LAB carrier because of the capacity of providing LAB growth. The growth in SDs was proportional to the mixture ratio (2:1) (mayonnaise: F. PBMAs). There were

approximately 10^7 CFU/ml bacteria in all types of salad dressing. SD 1, which included F. PBMA1, fermented by only yogurt isolates, had slightly lower bacterial counting results than others at the end of shelf life. According to the results, LAB count was supported and slightly increased during the shelf life. This could be caused by the sucrose content in mayonnaise. SD 3 was the salad dressing that LAB growth was the most in it according to the counting results of both MRS and M17.

In Fig. 3.13 and Fig. 3.14, results present as the mean values of two parallel counting (10^7 CFU/ml) for both replications. Counting was carried out for 10^{-5}, 10^{-6}, and 10^{-7} dilutions and their results were consistent. Also, the results that are shown in Fig. 3.13 and Fig. 3.14 are given in Appendix C.7 in a table format.

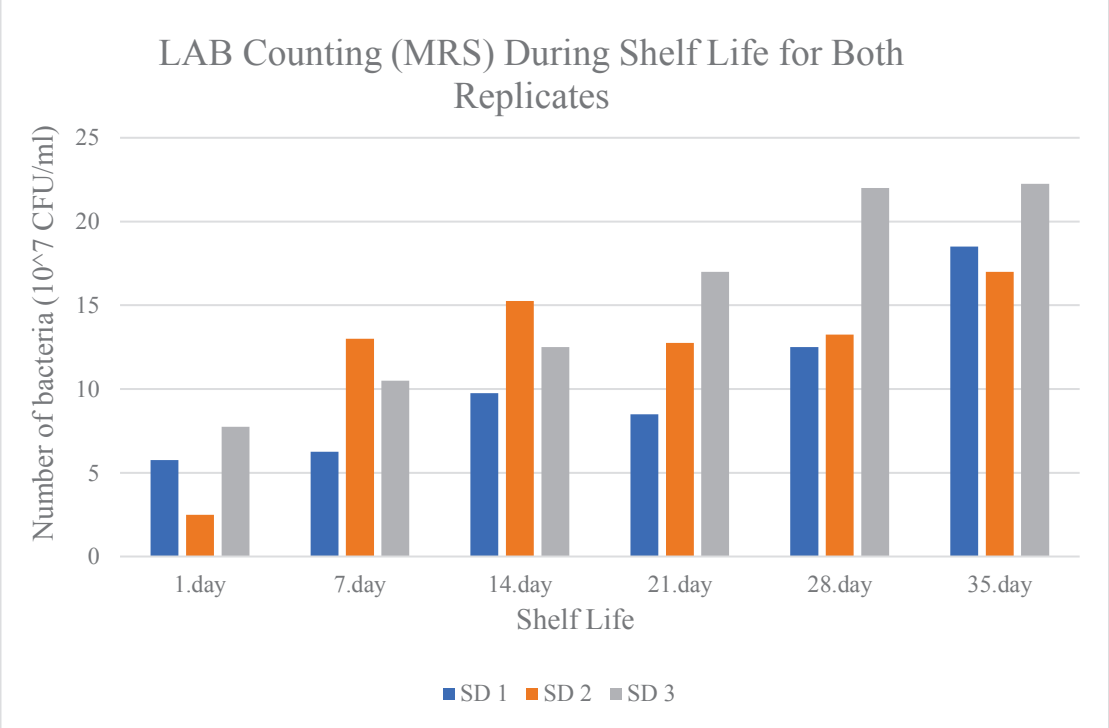

Figure 3.13. LAB counting in MRS during shelf life

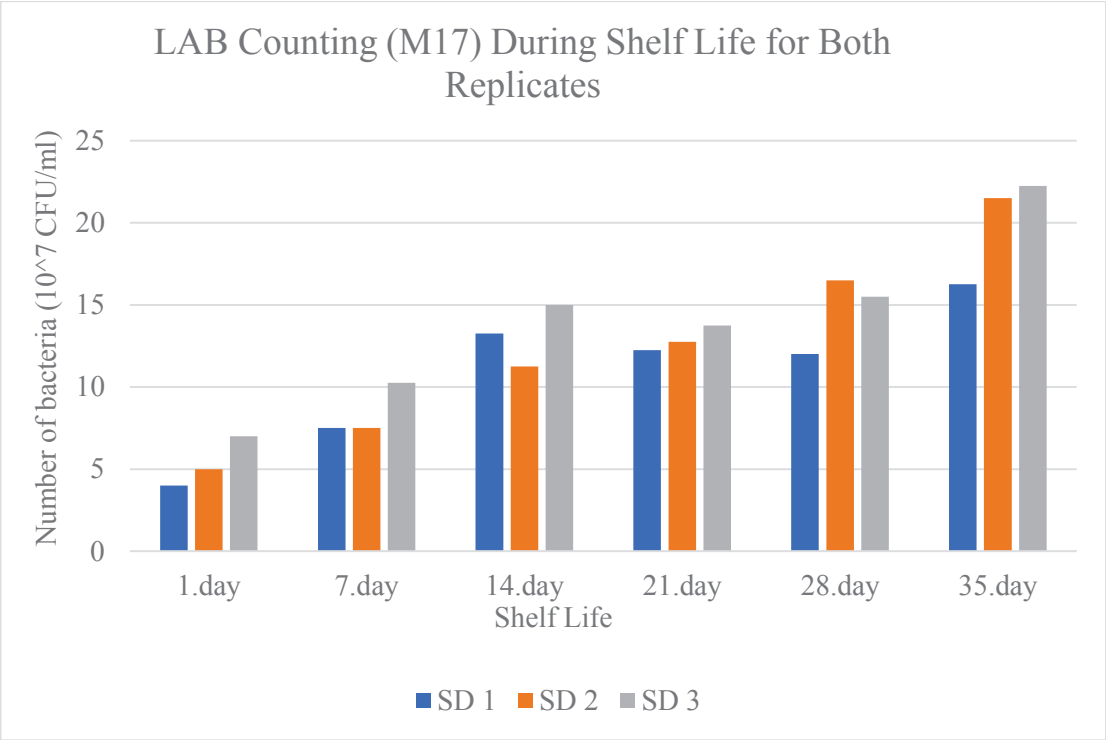

Figure 3.14. LAB counting in M17 during shelf life

3.3. pH Determination

The pH of CP milk alternative and PBMA was 6.6, AQF and vinegar were 6.0 and 3.0 respectively on average. The pH of CP AQF was stated as 6.26 in the study of He, Meda, et al. (2021). pH of AQF is one of the factors related to emulsion stability (Lafarga et al. 2019). Optimum growth pH of lactic acid bacteria was around 6.0, specifically 5.8-6.0 for *Lb. bulgaricus* and pH 6.5 for *St. thermophilus* (Rault, Bouix, and Béal 2009). Therefore, CP milk and PBMA were favorable in terms of pH for using bacteria. While pH values of CP pre-culture were almost the same during the fermentation and before the inoculation into PBMAs (Appendix B.8), pH values of F. PBMAs were slightly different for two batches, 4.7 and 5.1-5.5 (Appendix B.9). However, the pH difference of F. PBMAs between two batches did not cause a huge difference for salad dressings. This situation could be caused by the mixing ratio of F. PBMAs with mayonnaise. pH of mayonnaise ranged from 3.9 to 4.05. pH of SDs slightly decreased because of the slight increase in microbial load and was generally stable around 4.1-4.3 during the shelf life (Appendix B.10). pH of F. PBMA and SDs during the fermentation and the shelf life are shown in Fig. 3.15 and 3.16.

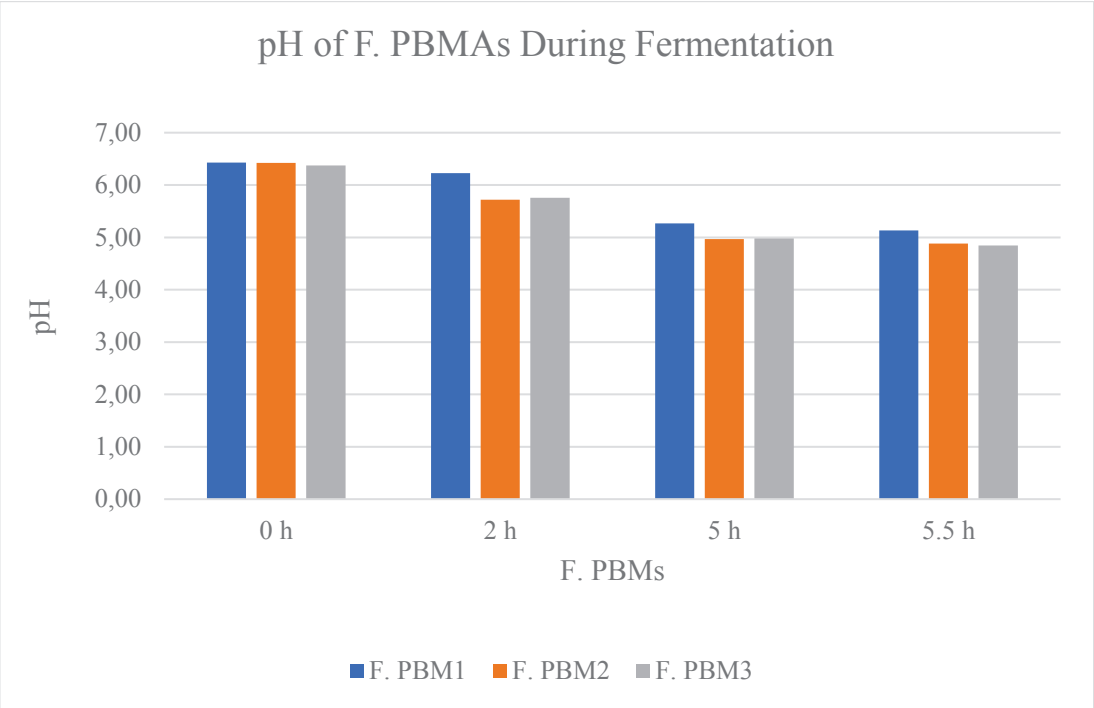

Figure 3.15. Fermentation time vs pH for F. PBMAs

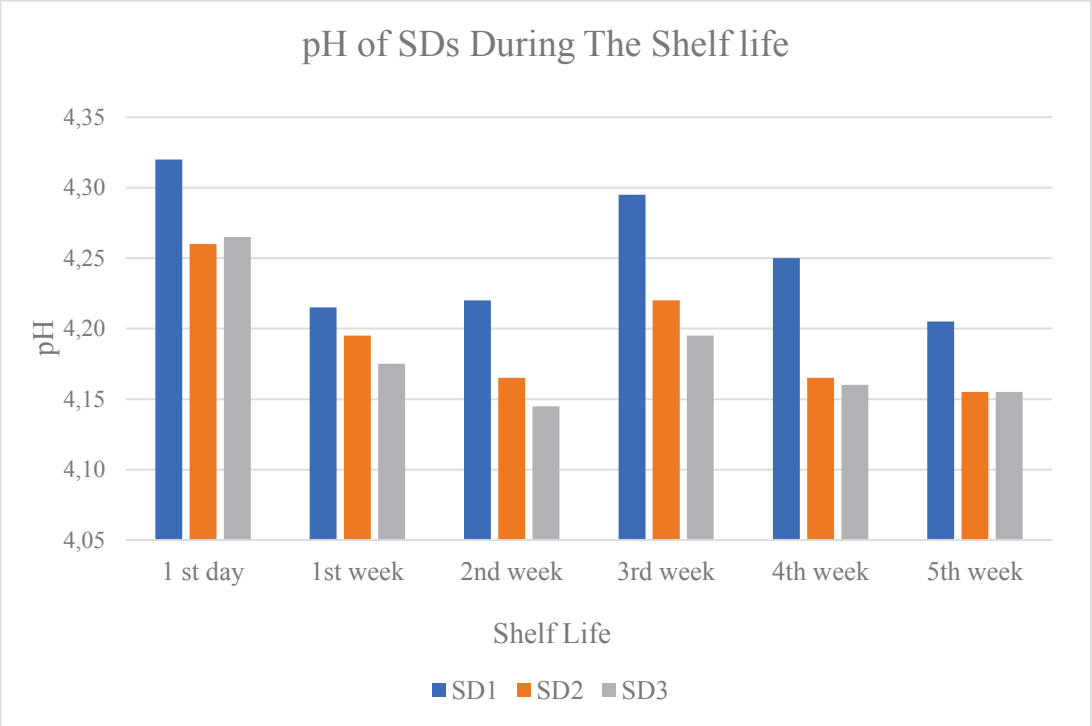

Figure 3.16. pH of SDs during the shelf life

In the study of Madjirebaye et al. (2022), tolerance of LAB, isolated from soybeans and fermented dairy products, to acid stress and bile salts was investigated. According to the results of mentioned study, two strains that were belonged to *St. thermophilus* and *Lb. plantarum* survived at pH 2.5 and bile salt at 0.5%. pH level below 4.0 is helpful to keep mayonnaise and salad dressing safe while inhibiting the growth of pathogenic microorganisms (Angelis et al. 2022). Inhibition of the growth of foodborne pathogens, such as *Escherichia coli, Clostridium botulinum,* and *Salmonella* can be realized under pH conditions lower than 4.0. While foodborne pathogens, such as *L. monocytogenes* and *Salmonella*, are caused by generally unpasteurized eggs in those types of products, use of pasteurized egg is chosen as a further precaution in commercial production (Smittle 2000). In this thesis study, AQF were kept under aseptic conditions until the preparation of mayonnaise. Since mayonnaise was pasteurized and the mixing step with F. PBMA was performed in again aseptic conditions, there was no unwanted bacterial growth in the SDs, although their pH levels were slightly higher than 4.0. Also, storage temperature is another effective factor for that.

3.4. Determination of Brix Value and Titratable Acidity (TA%)

Acids are one of the factors that affects the quality or flavor of foods, however it cannot be evaluated alone. Sugar content contributes to reducing the tartness of acids. Thus, brix/acid ratio is commonly used for a better prediction about flavor impact of acids than only the use of acid or brix (Tly and Sadler 2017). While brix/TA values are shown in Table 3.22, brix, and TA values of mayonnaise, PBMA, F. PBMAs and salad dressings were stated in Appendix D.

Table 3.22. Ratio of Brix/Titratable Acidity based on mean values of measurements

	1 st day	**1st week**	**3rd week**	**5th week**
Mayonnaise	5.26	5.24	5.52	6.74
SD 1	1.68	1.45	1.74	1.67
SD 2	1.93	1.57	2.29	1.69
SD 3	1.64	1.86	2.13	2.10

According to brix values, it can be basically said that sugar content was lower in the production batch which had lower pH value. That's why standard deviations of brix values of salad dressings were high. The brix of PBMA was 2.2, F. PBMA1, 2, and 3 were 0.45, 0.75, and 0.35, respectively. It can be said that sugar content decreased after fermentation as it is expected. The brix of mayonnaise, SD 1, 2, and 3 ranged from 16 to 19, 5.5 to 6.8, 5.5 to 8, and 6.6 to 8.05, respectively.

Besides that, titratable acidities of mayonnaise, SD 1, and SD 2 continued stable except the 5th week of storage. In the last week, the TA of mayonnaise decreased and TA values of SD1, and 2% increased. TA of SD 3% was regularly decreased during the shelf life. Average TA of mayonnaise and SDs were 3.0% and 4.0%. According to the study of Smittle (2000), TA in the water phase of some foodborne pathogens including *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on mayonnaise and salad dressings can differ from 0.65 to 1.72%. Also, it was reported that some strains of the mentioned pathogens can survive at 0.1% TA for an experimental mayonnaise. There were some dressings that have 0.43 to 5.25% TA as acetic in the water phase and pH 2.6 to 4.4 as extreme examples between the typical manufactured products in the US. In a study about full-fat mayonnaise, TA values ranged from 0.84 to 0.95 and the values of pH and TA are inversely proportional (Safitri, Evanuarini, and Thohari 2019). Another study related to low-fat mayonnaise supported this finding, pH, and TA% of the samples ranged from 3.9 to 4.5 and 0.85 to 0.6, respectively (Ataie, Shekarabi, and Jalili 2019).

3.5. Protein Analysis

While consumption of other macronutrients is adequate, protein intake of vegans is slightly under the Reference Nutrient Intake (Bakaloudi et al. 2021). The Kjeldahl method was carried out to determine the protein content in the samples (Table 3.23). The experiment was carried out based on grams as a unit which correspond to nearly the same amount ml of samples.

In literature, protein contents were determined as 2.38-9.21% for a functional low fat real mayonnaise (Ataie, Shekarabi, and Jalili 2019), and crude proteins ranged from 0.16 to 0.88% for six commercial salad dressings which were produced using egg yolk in Chinese market (Yin et al. 2022). The protein content of canned chickpea AQF was stated

as 1.27±0.02 and 1.21–1.72 in 100g (Raikos, Hayes, and Ni 2019; He, Meda, et al. 2021). Also, protein contents of AQF (from chickpea jars) and egg yolk were determined as 1.26±0.05 and 16.12±0.47 (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022). In comparison with egg yolk, AQF contains less amino acid and protein contents. Most aquafaba proteins can be classified as heat soluble hydrophilic species and heat stable (He, Meda, et al. 2021).

Table 3.23. Protein content of Salad Dressings and Mayonnaise

Sample	% Nitrogen	% Protein
Mayonnaise	0.043±0.011	0.266±0.071
SD 1	0.117±0.009	0.728±0.059[A]
SD 2	0.098±0.002	0.610±0.015[A]
SD 3	0.110±0.111	0.689±0.693[A]

[a]Results were expressed as mean ± standard deviation from 3 measurements (n = 1).

[b]The same uppercase letters in the same column mean that the samples are not significantly different (α=0.05).

As it was mentioned in the introduction part, legumes are rich in proteins. The mentioned protein values could not be reached in the SDs because of the milk obtained, and fermentation steps carried out. In a study about fermented chickpea and coconut beverage, protein content of the samples with different sugar amounts ranged from 1.13 to 1.27% (Mesquita et al. 2020). In another study, protein concentrations of sprouts of lentil, chickpea, and mung bean were determined as 2.63%, 2.19%, and 2.54% using the same method. . Protein content of 2.45% was stated as a mean value for those three pulses (Rizvi et al. 2022). In the study of (de Angelis et al. 2022), protein contents of vegan CP-based salad dressings prepared using CP flour and pea protein concentrate were investigated while commercial salad dressing was used as a control with 2.2% protein amount. The protein contents of developed salad dressings ranged from 4.26 to 5.16%.

In this thesis, the protein contents were not exactly comparable with the literature. However, the results demonstrated that using fermented plant-based milk alternative slightly contributed to an increase in protein amount in vegan mayonnaise. The protein contents of salad dressings were not significantly different ($p < 0.05$). To improve the

protein content, protein hydrolysates can be effectively used. Proteins of legumes have been used to obtain protein hydrolysates due to their availability and high nutritional value. According to the reported studies, enzymatic hydrolysis could be carried out to produce protein hydrolysate from lentil, chickpea, mung bean, soybean, and some other pulses. Legume protein hydrolysates are good sources of amino acids (Tawalbeh, Ahmad, and Sarbon 2022). In the study of Boeck, Sahin, et al. (2021), a market review was carried out on plant-based yogurt alternatives that sold in 16 different countries and it was reported that eight of 78 reviewed alternative products contain protein isolates in order to increase protein content. Also, according to the results of Boeck, Zannini, et al. (2021), which studied the production of a plant-based yogurt alternative using lentil protein isolate, protein content of the product was found equal to the protein content in dairy milk.

3.6. Emulsion Stability Index (ESI) and Emulsifying Activity Index (EAI)

ESI can be defined as a measurement of emulsion stability over time (Buhl, Christensen, and Hammershøj 2019). The ESI is a parameter that represents a turbidity decrease in a diluted emulsion over time and changes based on the sedimentation, coalescence, creaming, and flocculation resistance of proteins. EAI can be defined as an area of an oil/water interface that is stabilized per unit weight of protein (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022). Some studies in literature, emulsion stability (ES%) was preferred to be used. ES of full fat real mayonnaise fortified with ginger extracts was determined 89-94% while commercial mayonnaise showed 98.5% emulsion stability (Safitri, Evanuarini, and Thohari 2019). ES of CP AQF in different pH and CP: water ratios were investigated and ES of AQF was found between 0 and 76.3%. Besides that, emulsion capacity (EC) was determined 3.9-72.3%. Additionally, it was indicated that lower pH and CP: water ratio, which allows higher amounts of protein in AQF, values contributed to obtaining good emulsifying abilities. It was predicted that EC and ES values were optimum when pH and CP: water ratio values were 3.5 and 1:1.72, respectively (Lafarga et al. 2019). The relation between pH and emulsifying activities was also investigated by (Buhl, Christensen, and Hammershøj 2019), higher ESI value was observed at pH level higher than 6.0 for centrifuged AQF based emulsions.

ESI and EAI were determined during each two weeks of the shelf life as shown in Table 3.24. According to those ranges, EAI of mayonnaise was lower with addition of salad dressings and their values were lower than the literature. This situation could be caused by AQF production technique. While there was a significant difference between the 1st and other weeks of the EAI values of SD 1, there was no significant difference for SD 2 and SD 3 between the 1st and 3rd week of shelf life. The EAI values of salad dressings were consistent comparing to each other ($p > 0.05$).

Table 3.24. Emulsion Stability Index (ESI), Emulsifying Activity Index (EAI), and Protein Content of Salad Dressings and Mayonnaise

	1.week	**3.week**	**5.week**	**Protein Content (%)**
		EAI		
Mayonnaise	0.36±0.02^{A}	0.34±0.03^{A}	0.36±0.00^{A}	0.266±0.071
SD 1[a]	0.18±0.04^{A}	0.12±0.01^{B}	0.12±0.05^{B}	0.728±0.059
SD 2[a]	0.18±0.02^{A}	0.14±0.01^{B}	0.18±0.01^{A}	0.610±0.015
SD 3[a]	0.17±0.05^{A}	0.15±0.04^{B}	0.18±0.02^{A}	0.689±0.693
		ESI		
Mayonnaise	26.73±0.02^{A}	36.18±0.04^{A}	26.96±0.01^{A}	
SD 1[a]	29.36±0.08^{A}	28.45±0.02^{A}	31.76±0.14^{A}	
SD 2[b]	36.76±0.23^{A}	32.93±0.01^{A}	76.17±0.12^{B}	
SD 3[b]	30.60±0.14^{A}	31.45±0.00^{A}	50.56±0.05^{A}	

[a]Results were expressed as mean ± standard deviation from 2 measurements and 2 replications (n = 2).

[b]The same uppercase letters in the same row mean that the samples are not significantly different. The different lowercase letters in the same column mean a significant difference between samples (α=0.05).

In the study of Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak (2022), ESI and EAI values of egg-yolk and AQF were compared and EAI value of AQF (13.75 m^2/g) was greater than the EAI of egg yolk (1.78 m^2/g). However, the calculated EAI of egg yolk was lower than the values in literature which differs from 24.5 to 30.5 m^2/g. ESI value of AQF was significantly lower than the value of egg yolk, 20.92 min and 2385 min, respectively. If the EAI value of AQF proteins is higher, it can be based on the higher

solubility and less compact structure combination that develops the formation ability of interfacial membranes around the oil droplets (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022). In literature, besides EAI and ES values of AQF, which were determined as 1.1-1.3 m^2/g and 71 to 77% (He et al. 2019), the values were ranging from 12 to 38.6 m^2/g and 15 to 25 min, respectively (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022).

ESI values of mayonnaise and salad dressings were lower than the value of regular mayonnaises however comparable with the previously published values about AQF. ESI value of mayonnaise was not significantly changed over time ($p > 0.05$). While the ESI value of SD 2 was significantly changed in the 5^{th} week, there were no significant differences in SD 1 and SD 3 over time. An experimental error could have occurred during the ESI value determination of SD 2 in the 5^{th} week. There was a significant difference between the values of SD 1 and SD 2 in the context of ESI. There was a phase separation at the bottom of the salad dressings while there was not in the mayonnaise sample. This situation could occur because of the microbial load in salad dressings.

3.7. Moisture and Ash Content

Moisture and ash contents of salad dressings and mayonnaise were measured in the 3^{rd} week of shelf life (Table 3.25). In literature, moisture content of real reduced fat mayonnaise was determined as 18.40-44.34%, while ash was stated as 0.88-1.23% (Ataie, Shekarabi, and Jalili 2019). In the study of Yin et al. (2022), commercial salad dressings, that were investigated to understand their compositions, had contained 21-51% of moisture and 1.24-2.63% ash. Ash and moisture contents of AQF were determined as 0.44% and 94.97% (Raikos, Hayes, and Ni 2019). In the study of Mesquita et al. (2020), moisture and ash contents of fermented vegan beverages were within the range of 83-91% and 0.30-0.33%, respectively. In another study, ash content of vegan salad dressings ranged from 1.45 to 1.56%, and commercial salad dressing which was used as a standard contained 2.40% ash (Angelis et al. 2022).

While the moisture of mayonnaise is comparable, its ash content is a little bit lower. Mayonnaise based salad dressings gained moisture content by the addition of F. PBMAs. The ash contents of SDs are lower than a commercial salad dressing.

Table 3. 25. Moisture and ash content (%) of mayonnaise and salad dressings

	Moisture content (%)	Ash (%)
Mayonnaise	23.18±0.89	0.63
SD 1	47.87±1.49	0.55
SD 2	47.83±1.63	0.54
SD 3	48.11±0.10	0.50

Results were expressed as mean ± standard deviation for moisture content (n = 2)

3.8. Mineral analyses

The micronutrient intakes of vegan diets were compared to the recommendations of WHO by Bakaloudi et al. (2021). Calcium and iodine intakes were found to be lower than non-vegan diets and inadequate, while the intake of iron is higher than other types of diet. Besides that, sodium intake exceeds the Reference Nutrient Intake. Mineral content of salad dressings and their base materials are shown in Fig. 3.17 and the data were reported as a table format in Appendix E. According to EFSA (2017), the population reference and adequate intakes of minerals were stated for female adults in order to state the minimum required intake for adults (the reference intakes during the pregnancy is neglected). Reference intakes of calcium and zinc are 950-1000 and 7.5-12.7 mg/day. Adequate intake of phosphorus, manganese, magnesium, potassium, iron, and copper are 550, 3, 300, 3,500, 11, and 1.3 mg/d, respectively.

In a study about salad dressings and mayonnaise in the Malaysian market, it was stated that there was a high amount of Ca in the salad dressings and mayonnaise. Sodium amount was 28% of the recommended sodium intake allowance by U.S. (2.4 g/day). The iron, copper, zinc, and magnesium concentrations are similar for mayonnaise and salad dressings (Abd Rashed et al. 2017).

The calcium amount of PBMA seems to be decreased after pasteurization and mayonnaise meets the 2:3 of the calcium amount for salad dressings. Measured mineral contents of mayonnaise are higher than PBMA except Ca, P, and Mg. Besides that, K, Mg, P, and Ca (except SD 3) concentrations increase with the addition of F. PBMAs. Zn concentration of SD 2 was accepted as an error. SD1 and SD2 seem to contain higher Ca than SD3, this could be caused by an experimental error. As it was mentioned in the

introduction part, the absorption of dietary Ca suppresses the renin and probiotics can have a role in the Ca absorption thus, Ca amount of the products can be one of the factors that affects the ACE-I activity.

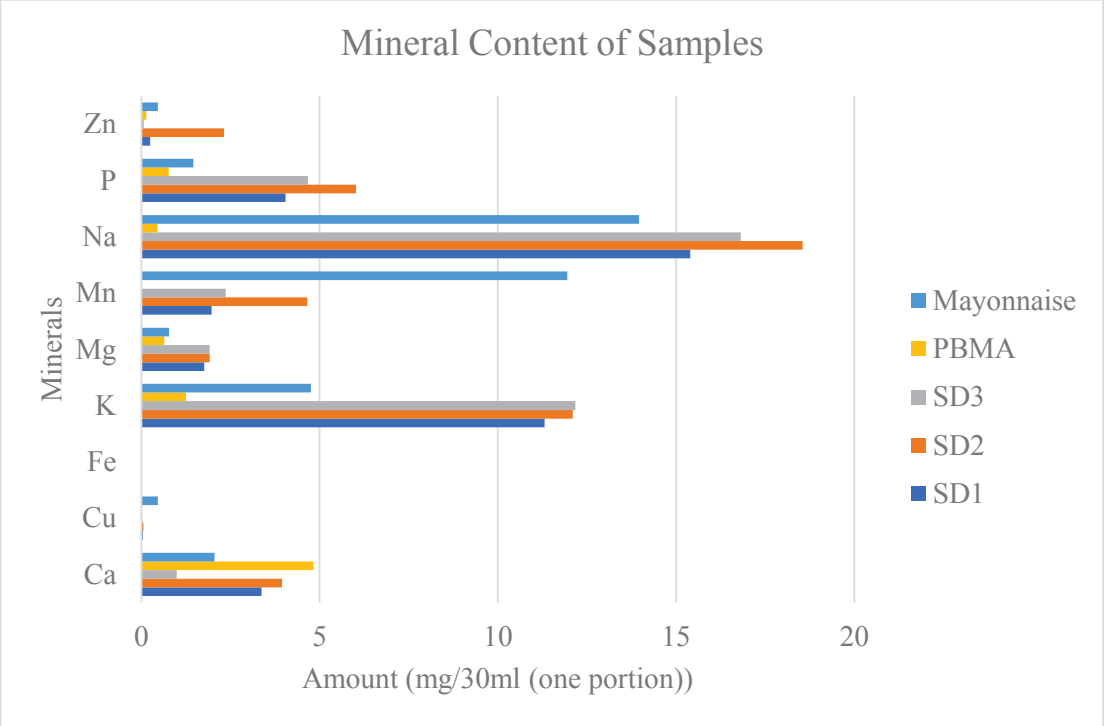

Figure 3.17. Mineral content of PBMA, mayonnaise, SD1, SD2, and SD3

While Cu, K, and Mg amounts were similar, the amounts of Mn, P, and Na were higher in SD 2 than other salad dressings. There was nearly no Cu content in salad dressings and no measurable iron content in the products (below 50 ppb) . Sodium was the most present mineral content (510-620 mg/kg) in salad dressings because of the salt in mayonnaise formulation. One of the targets of WHO is salt reduction because of excessive consumption of it, around twice of the maximum recommended intake level, worldwide. Salt is a main source of sodium in diets and high consumption of sodium (more than 2g/day or 5g of salt/day) causes high blood pressure, so increases the risk of heart diseases (WHO 2020). 1 kg of salad dressing corresponds to ~28.25% of the daily sodium intake. However, 30 g can be recommended as a portion and the Na amount in it meets 1.7% of the daily intake. Insufficient intake of potassium (less than 3.5 g/day) is also another factor that contributes to high blood pressure (WHO 2020). Chickpea and

lentil are rich in potassium (Iqbal et al. 2006). The potassium content of salad dressings ranges from 377 to 405 mg/kg which meets on average 0.34% of daily potassium intake per portion.

3.9. Color Analysis

Since mayonnaise is a high fat food, oxidative deterioration is a possible problem that negatively affects the nutritional value, aroma, color, flavor, and color of the food (Raikos, Hayes, and Ni 2019). Color is a crucial factor that has an impact on the willingness of the consumers to taste a food product (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022). While the results of color measurements were reported in Table 3.26, the color of the mayonnaise, F. PBMAs, and salad dressings were shown in Fig. 3.18. The addition of F. PBMAs caused a decrease in the WI, therefore the WI of mayonnaise was greater than the salad dressings. The color parameters of salad dressings were close to each other. There was a slight decrease in the WI between the 1st and 5th week of shelf life in all samples and the highest decrease was observed in the SD 3.

Table 3.26. Color parameters of mayonnaise and salad dressings in 1st and 5th weeks of shelf life

	1st week			
	L*	a*	b*	WI
Mayonnaise	79.82±0.03	(-6.72)±0.01	9.57±0.05	76.67±0.0
SD 1	77.85±0.13	(-6.44)±0.05	9.46±0.19	75.06±0.05
SD 2	78.35±0.08	(-6.54)±0.01	9.78±0.02	75.35±0.07
SD 3	78.55±0.11	(-6.46)±0.0	9.10±0.07	75.81±0.0
	5th week			
	L*	a*	b*	WI
Mayonnaise	77.92±0.09	(-6.36)±0.01	7.51±0.04	75.81±0.04
SD 1	77.13±0.09	(-6.25)±0.01	8.89±0.05	74.67±0.08
SD 2	76.15±0.01	(-6.16)±0.02	8.33±0.02	73.99±0.02
SD 3	76.34±0.0	(-6.09)±0.04	8.32±0.08	74.19±0.14

Results were expressed as mean ± standard deviation (n = 2)

(a) (b) (c)

Figure 3.18. Mayonnaise (a); F. PBMAs (b); and SDs (c)

3.10. Total Phenolic Content Analysis

The phenolic content as well as the antioxidant activity depends on the different factors such as raw materials and physicochemical characteristics (Romeo et al. 2021). One of the various nutrients that was included in AQF is the phenolic compounds. Those compounds influence solubility, emulsifying and foaming properties of polysaccharides and proteins. Flavonoids which are found in legume can show an effect on the emulsifying ability to oil-water solutions. Moreover, the gelation property of AQF might be associated with protein-polyphenol interaction (He, Meda, et al. 2021). Phenolic contents were found around 7500 mg L^{-1} by de Bruno et al. (2021) for phenolic extract enriched vegan mayonnaise. Total phenols of AQF was found to be 6.5 mg GAE/g (Raikos, Hayes, and Ni 2019). Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak (2022) mentioned a study that investigates the relation of the ESI and EAI and gallic acid concentrations for emulsions of lentil protein isolate–phenolic solutions. In the mentioned study, ESI and EAI were inversely proportional with the phenolic extracts and gallic acid concentrations obtained from onion skin.

The phenolic contents of mayonnaise and salad dressings were reported in Table 3.27. F. PBMAs could not contribute to the phenolic content of mayonnaise. The phenolic contents of salad dressings were significantly different over time, while mayonnaise had no significant phenolic content difference during the shelf life. The phenolic contents of vegan mayonnaise and salad dressings were lower than the values in the literature. In this case, it can be said that the phenolic content of mayonnaise is also associated with choosing oil type. The use of only AQF and plant-based milk alternative are not sufficient to obtain a high phenolic content.

Table 3.27. Phenolic Contents (mg gallic acid/L) of mayonnaise and salad dressings during the shelf life

	Mayonnaise[a]	SD 1[a]	SD 2[a]	SD 3[a]
1st week	3295±471^{A}	3485±51^{A}	4934±301^{A}	2867±54.87^{A}
3rd week	2383±90^{A}	2091±461^{B}	1794±283^{B}	2118±256^{B}
5th week	3264±199^{A}	3821±61^{A}	2405±51^{B}	2318±181^{AB}

[a]Results were expressed as mean ± standard deviation from 3 measurements (n = 1).

[b]The same uppercase letters in the same column mean that the samples are not significantly different. The same lowercase letters in the same raw mean no significant difference between samples (α=0.05).

3.11. Antioxidant Activity (DPPH, ABTS) Assay

To evaluate the antioxidant activity of the salad dressings and mayonnaise were determined performing DPPH and ABTS assays and results were reported in Table 3.28. Also, Trolox standard curve was stated in Appendix F.

Table 3.28. Evaluation of antioxidant activity of mayonnaise and salad dressings during the shelf life

	Mayonnaise[a]	SD 1[a]	SD 2[a]	SD 3[a]
ABTS (µM Trolox/ml)				
1st week	n.d.	n.d.	n.d.	n.d.
3rd week	542±145^{A}	432±172^{A}	446±236^{A}	427±216^{A}
5th week	554±312^{A}	573± 273^{A}	394±270^{A}	575±320^{A}
	Mayonnaise[a]	**SD 1[b]**	**SD 2[b]**	**SD 3[ab]**
DPPH (% inhibition)				
1st week	64.31±3.52^{A}	51.72±2.14^{A}	52.89±2.97^{A}	65.24±0.84^{A}
3rd week	54.27±0.00^{B}	41.43±6.84^{A}	50.12±1.90^{A}	48.47±1.90^{B}
5th week	74.12±11.98^{A}	52.11±6.08^{A}	50.76±14.41^{A}	61.55±6.92^{AB}

[a] Results were expressed as mean ± standard deviation from 3 measurements (n = 1).

[b] n.d. means the values could not be detected.

[c] The same uppercase letters in the same column mean that the samples are not significantly different. The same lowercase letters in the same raw mean no significant difference between samples (α=0.05).

Romeo et al. (2021) enriched the real mayonnaise by the addition of phenolic extracts and investigated the antioxidant activity applying ABTS and DPPH assays during the storage. While inhibition values ranged from 27.5 to 70.3%, a decrease was observed over time. The results of ABTS assay were as 6 mmol Trolox/g on average and changed with the effect of time (Romeo et al. 2021). In the study of de Bruno et al. (2021), vegan mayonnaise, which was enriched with phenolic extracts, was addressed and the results of ABTS and DPPH were on average 27,000 and 1000 μmol Trolox ml^{-1}, respectively. Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak (2022) found the DPPH and ABTS values of AQF as 437 and 2097 µmol Trolox/100g, respectively although the values in the literature were lower than those values and ranged between 0.15-0.38 µmol Trolox/g.

The DPPH assay is generally more applicable for hydrophobic antioxidant systems, while the ABTS assay can be carried out for both lipophilic and hydrophilic systems. Since AQF is a hydrophilic system, water-soluble antioxidants were dominant so that the ABTS value was higher than the DPPH value (Włodarczyk, Zienkiewicz, and Szydłowska-Czerniak 2022).

The results of ABTS assay in the first week were not usable. However, the results of ABTS and DPPH were consistent with each other in the same weeks ($p > 0.05$). The ABTS results of mayonnaise, and salad dressings were not significantly changed over time. Also, DPPH values of SD 1 and SD 2 were not significantly changed during time. Besides that, there was a significant difference in the DPPH values of mayonnaise in the 3rd and 5th weeks, while a significant difference was observed between the values of SD 3 in the 1st and 3rd weeks ($p < 0.05$). In the ABTS assay, there was no significant difference between mayonnaise and salad dressings, while SD 1 and SD 2 were significantly different, and SD 3 was slightly different than mayonnaise in the DPPH assay. The DPPH values were comparable, however the ABTS values were lower than the literature. According to literature, DPPH values were lower than the ABTS values for AQF or AQF based emulsions as it was in this thesis study. In these conditions, ABTS method would be also preferred for the detection of antioxidant activity which could be originated from plant-based milk alternative. Consequently, the antioxidant activity was detected however, not much as in the literature as well as the phenolic content.

Antioxidant ability of LAB is one of the reasons that they are a focus of interest. In a study in this topic, the selected LAB strains were investigated for their probiotic and

antioxidant potentials, and it was found that they have a strong potential to be used as new probiotics with antioxidant effects (Kim et al. 2022). In a study of Degrain et al. (2020), different strains of LAB were used in fermentation of nightshade leaves, and it was reported that the greater effect on the phenolic content and antioxidant activity was strain dependent as well as dependent on the matrix and a *Lb. plantarum* strain had a greater potential in that context. Therefore, it can be said that LAB strains have an effect on antioxidant activity and using different bacterial strains, which both potentially provide high proteolytic and antioxidant activities, might be considered and investigated for further studies.

3.12. OPA Analysis

Determination of the proteolytic activity that is caused by LAB can be helpful to understand the effects of them on the product characteristics such as flavor and bitterness (Garbowska, Pluta, and Berthold-Pluta 2020). Also, proteolytic activity is directly proportional with ACE-I activity, it is required a strong proteolytic activity to produce antihypertensive peptides (Pihlanto, Virtanen, and Korhonen 2010). In the study of (Pescuma et al. 2010), *Lb. delbrueckii* subsp. *bulgaricus* strain was found the most proteolytic strain with 626 μg Leu/ml, while the mixed stater LAB cultures (selected strains of *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and *St. thermophilus*) showed high proteolytic activity (484 μg/ml Leu) during the fermentation of a dairy-based product, whey protein concentrate with 35% protein content which had a low OPA value (82.3 μg/ml) in its unfermented form. In the study of (Donkor et al. 2005), the proteolytic activity of yogurt cultures and some selected probiotic strains were determined using soy yogurt during the storage time and the values increased at the end of storage.

The proteolytic activity of the samples was measured performing OPA analysis. This assay was carried out with five parallel in order to minimize the fluctuation in the absorbance values obtained from Varioskan and results were reported in Table 3.29. Standard curve was drawn using L-leucine and stated in Appendix G (R^2=0.9981).

Table 3.29. The results of OPA analysis in terms of mg L-leu/ml during the shelf life

	1st week	3rd week	5th week
SD1	0.322±0.05^{Ab}	0.297±0.01 ^{Ab}	0.312±0.01 ^{Ab}
SD2	0.458±0.03 ^{Aa}	0.442±0.04 ^{Aa}	0.485±0.01 ^{Aa}
SD3	0.541±0.01 ^{Aa}	0.480±0.0 ^{Aa}	0.516±0.02 ^{Aa}
Mayonnaise	0.110±0.01		

[a]Results were expressed as mean ± standard deviation from 5 measurements and 2 replications (n = 2).

[b]The same uppercase letters in the same row mean that the samples are not significantly different. The different lowercase letters in the same column mean a significant difference between samples ($\alpha = 0.05$).

In this thesis, the distributions of obtained data were found normal. The proteolytic activities of salad dressings were not significantly different over time ($p > 0.05$). Mayonnaise had a lower proteolytic activity and was reported as a control sample. SD 1, which was fermented by only yogurt isolates, showed the lowest proteolytic activity and significantly different than SD 2, and SD 3 ($p < 0.05$). The results are comparable with the literature. It can be said that the proteolytic activities of the LAB were comparable for SD 2 and SD 3, while the value for SD 1 was slightly lower than the values measured for LAB fermentation using dairy products in the literature.

3.13. ACE Inhibition (ACE-I) Activity Assay

The pH of F. PBMAs, salad dressing samples, and mayonnaise were arranged to 8.3 using 5-10 µl, 20-30 µl, and 50 µl NaOH (2.5 M). The assay buffer was included instead of samples as a control. HHL, sample and buffer mixture were tested to remove the potential unwanted peak area in the HA peak for evaluating as a blank. There was no unwanted peak formation under the HA peak. Baseline separation of HA from HHL was achieved in 10.8 min. The ACE-I activities of samples were determined immediately after the samples were taken out from the cold storage; because approximately 2% decrease in ACE-I activity was observed in 5-7 hours when the samples were kept at room temperature. The results of the analysis were reported as mean values of two parallels and two replicates. Fig. 3.19 and Fig. 3.20 show the ACE-I activities of base materials and

salad dressings respectively and those results were stated as a table format in Appendix H. Hippuric Acid Standard Curve ($R^2 = 0.9999$) was also reported in Appendix H.

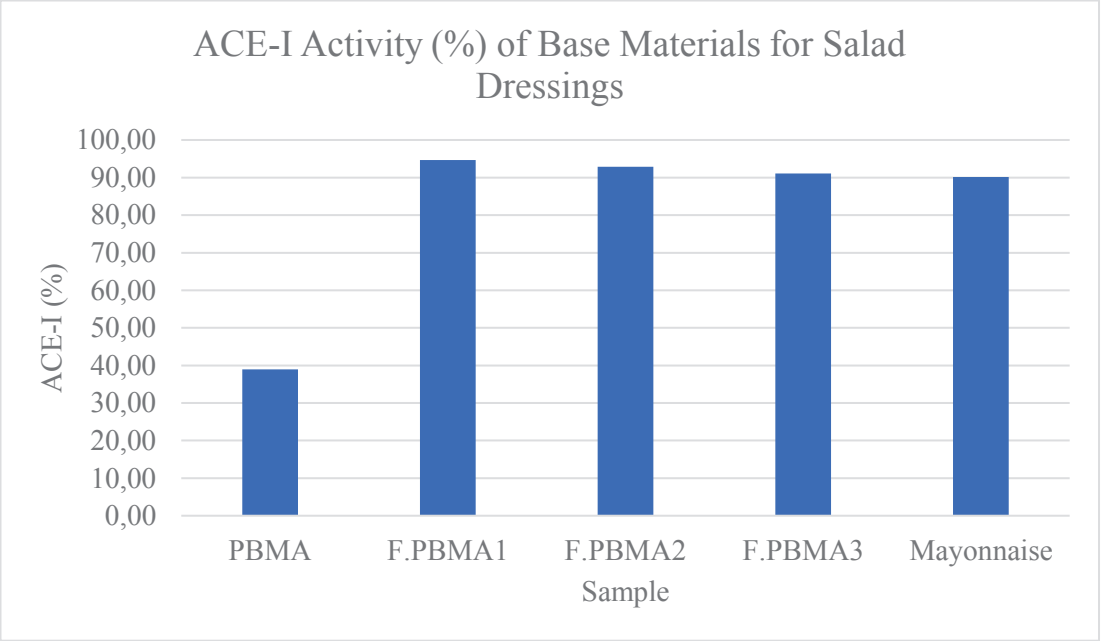

Figure 3.19. ACE-I Activity (%) of Base Materials for Salad Dressings

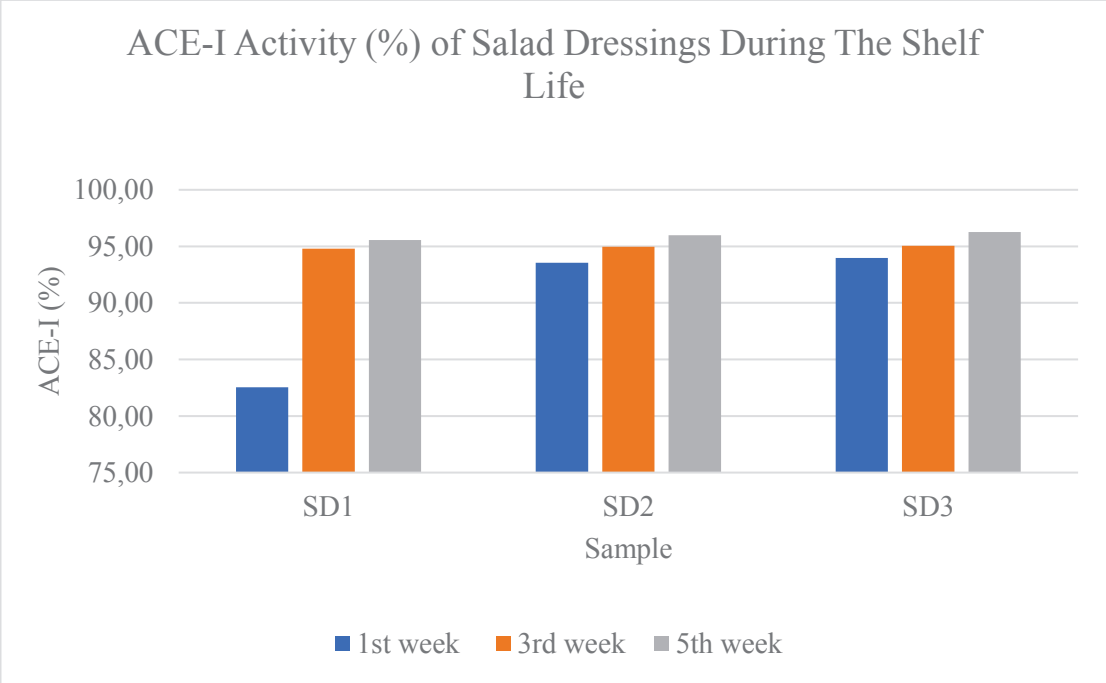

Figure 3.20. ACE-I Activity (%) of Salad Dressings During the Shelf Life

According to the results (Fig. 3.19), fermentation increased the ACE-I activity of PBMA (38.87%). According to the literature, a strain of *Lb. plantarum* inoculated in mung bean to produce probiotic foods, and more than 10^8 CFU/ml viable count was observed besides the significantly higher ACE-I activity after fermentation (Rasika et al 2021). In this thesis, the effect of LAB on the ACE-I activity was at a measurable level (more than two times of the PBMA value). There was a significant difference between F. PBMA 1 and F. PBMA 3. The ACE-I activity of F. PBMA 2 had similarities with both F. PBMA 1 and F. PBMA 3. Thus, it can be said that *Lb. bulgaricus* strain (bty 8b), which was contained in F. PBMA 2, had slightly higher ACE-I activity than *Lb. bulgaricus* strain (bty 69), which was involved in F. PBMA 3. However, there were no significant differences in the proteolytic activities of these two strains as mentioned before in section 3.12. OPA Analysis.

The ACE-I activities of SD 1 were significantly changed after the first week of shelf life. There were significant differences in the activities of SD 2 and SD 3 between the initial and the final weeks of the storage. The third week can be accepted as the time of the storage which the changes realized. The differences in SD 3 ($p = 0.018$) were more significant than SD 2 ($p = 0.04$) over time. As mentioned before in section 3.12, samples that had lower proteolytic activity showed lower ACE-I activity for the 1st week. However, this difference was not observed for the rest of storage time. In the study of (Donkor et al. 2005), ACE-I of soy yogurt which was produced using with the addition of probiotics was greater than the one using only stater cultures. In this thesis, there was a difference in the ACE-I activities of SDs similar to the study of (Donkor et al. 2005), while this relation was not observed for F. PBMAs.

The ACE-I activity of the mayonnaise sample was determined as 90%. After the addition of F. PBMAs, ACE-I activities increased about 3.5-6% during shelf life. It could be an experimental error in the first week's measurements of SD 1. As a positive control, 7% and 9% ACE-I activities were calculated for the 0.005 and 0.015 μM concentrations of captopril. Therefore, increasing values in mayonnaise because of the F. PBMAs can be accepted as considerable changes even if there were no statistically significant differences between the mayonnaise and salad dressings.

Pihlanto, Virtanen, and Korhonen (2010) investigated the antihypertensive effect of milk which was fermented by different strains of LAB and the results of ACE-I activity

ranged from 2 to 74%. The probiotic potential of some LAB strains that were isolated from fermented Greek dairy products were examined by Georgalaki et al. (2017). According to their results, the ACE-I activities of four lactobacilli and eight cocci, which were grown into skim milk, were accepted as strong (higher than 70%). Then, the ACE-I activity of two selected strains of *Lb. delbrueckii* subsp. *bulgaricus* and *St. thermophilus* were investigated in different types of milk (sheep, cow, and goat) and the activity was found dependable to the types of milk. Therefore, it can be said that the growth media affects the level of ACE-I activity.

The use of LAB in the fermentation of legumes has some limitations as mentioned in the introduction part. According to Maleki and Razavi (2020), one of the limiting factors is low proteolytic activity which affects the releasing bioactive peptides. Besides that, reduction of pH during the fermentation can influence the solubility of protein and phenolic compounds in legumes and causes the reduction of functional properties originating from bioactive compounds. Thus, LAB can be used with other microbial cultures to enhance the ACE-I activity. Also, many polyphenols and legume-derived peptides can contribute to the high ACE-I activity because they can act as ACE inhibitors (Penas et al. 2015). Therefore, it can be said that pH conditions during the fermentation and the phenolic content of the products can be considered when the ACE-I activity comparisons were made. The amount of polyphenol might be improved to obtain higher ACE-I activities. Also, legume proteins are considered significant sources for the isolation of bioactive peptides which can be potentially utilized for treating and preventing various diseases besides the improvement of protein content as mentioned in the 3.5 Protein Analysis. The enzymatic hydrolysis of legume proteins can be used effectively to release bioactive peptides with ACE inhibitory and antioxidant activity (Tawalbeh, Ahmad, and Sarbon 2022). Several studies reported the hypotensive effect caused by ACE-I activity of isolated hypotensive peptides originated from plant proteins which were processed by enzymatic hydrolyzation and fermentation (Shobako 2021). For further improvement in the ACE-I, plant protein hydrolysates can be used.

CHAPTER 4

CONCLUSION

In this thesis context, vegan food formulations, fermented plant-based milk alternative (F. PBMAs), mayonnaise producing with chickpea AQF, and vegan mayonnaise based-salad dressings (SDs) were developed. Fermentation was carried out using LABs which have the potential to show high proteolytic activity based on a previous selection. After obtaining chickpea: yellow split lentil: mung bean plant-based milk alternative (PBMA), 5±0.5 h of fermentation using mentioned LAB was found suitable for as far as preventing the phase separation, and off-odor caused by protein denaturation and legumes themselves. A vegan mayonnaise formulation was selected among eight products prepared applying two formulations with different production ratios of AQF and oil types based on pH and the capacity of supporting LAB load. Salad dressings were prepared using the selected vegan mayonnaise, and three types of F. PBMA, which were fermented by only yogurt isolates, and the separate additions of two other *Lb. bulgaricus* strains at the 2nd hour of the fermentation.

The originality of salad dressing products obtained in this thesis arises from their fermented nature. There were 10^7 CFU/ml LAB in the salad dressings during the shelf life. Quality characteristics of vegan mayonnaise and salad dressing products gave similar characteristics with regular mayonnaise and salad dressings. Also, comparable results were found with other vegan mayonnaise and salad dressings in the literature. The phenolic contents and antioxidant activities of the products were not that high and using oil type could be a factor for that. Present phenolic contents could mostly be originated from AQF. The proteolytic activities of the salad dressings were comparable to the values in the literature. ACE-I activities were at a measurable level and increased during the shelf life. Fermentation developed the ACE-I activity of PBMA (app. 40%). The ACE-I activities of F. PBMAs were detected as 93% on average. After the addition of F. PBMAs into mayonnaise (90%), ACE-I activities increased about 3.5-6% during shelf life.

Comparing the used LAB strains is a challenge. SD 1 that included only yogurt isolates combination; *Lb. bulgaricus* strain (bty 73) and *St. thermophilus* strain (cty 44) had significantly different or not from other salad dressings in different analyses. Besides

that, there were slight differences or no significant difference between SD 2 and SD 3, which included different *Lb. bulgaricus* strains, from analysis to analysis. For instance, SD 1 was different from SD 2 and SD 3 in terms of ESI while there was no difference between them in terms of EAI. SD 3 seemed to have a slightly higher antioxidant activity than SD 2 while a significant difference was not observed between those two SDs in terms of phenolic content, ESI, EAI, OPA, and ACE-I activity. However, there was a slight difference between ACE-I activity of F. PBMA 2 and F. PBMA 3, which were included in SD 2 and SD 3. Also, all SDs had almost the same pH, besides their similar microbiological counting results. This case may be investigated further in future studies.

Fermented salad dressings were obtained without addition of any preservatives/additives. Shelf life has been examined for 5 weeks, no contaminants and spoilage microorganisms have been detected. It can be said that storage time may be prolonged double considering pH values (approx. 4.2) of salad dressings. In addition to that, vinegar ratio can be increased, and mustard or different natural preservatives can be added into the obtained base salad dressing. Also, it can be predicted that the shelf life can be extended by using aseptic filling and vacuum packaging.

In this thesis, it investigated how a product rich in fat and containing salt can be consumed healthier in terms of hypertension. The ACE-I activity of PBMA was enhanced by LAB fermentation and vegan mayonnaise was enriched with those high value ingredients. Consequently, fermented vegan food formulations having ACE-I activity with potential antihypertensive effect have been developed.

As future prospects, carrying out sensory analysis would be helpful to be sure whether there is an off flavor because of the ingredients or not, and overall consumer acceptance. Protein hydrolysates can be effectively used for improving the protein content. Calcium amount and phenolic content might be enriched, and their effects may be investigated further considering their relations with ACE-I activity. *Lb. plantarum* might be investigated to be used for further similar studies because of the potentially high antioxidant and ACE-I activities, and the suitability of use in non-dairy matrices of its strains as it mentioned before. Finally, the fat and salt content of salad dressings might be reduced to the minimum within the scope of regulations.

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APPENDICES

APPENDIX A. McFarland Values of Using Bacteria for Fermentations in The Trials

Table A.1. McFarland values of using bacteria for fermentations in the trials

Bacteria	Bty 73	Cty 44	Bty 5	Bty 8b	Cty 41	Bty 71	Bty 8b	Bty 69
Pre-culture testing CP (1:6)	8.3-8.4	7.3-7.4	7.9	9.0	7.4-7.5			
1st trial CP (1:12) (for 20ml)	8.4-8.5	5.9	8.4	8.9	7.6			
1st trial (for 100ml)	9.5	7.2						
2nd trial (for CP & broths)	8.6 & 3.8	7.6 & 7.7						
3rd trial	8.9	8.0						
4th trial	8.4	7.7						
5th trial	9.1	7.8				7.9		
6th trial	8.4	7.7						
7th trial	8.7	7.4				9.0	8.4	7.9

APPENDIX B. pH Values of CP Pre-Culture and F. PBMAs During Fermentation

Table B.1. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives (100 ml) in the 1st trial

	0 h	4 h	5 h	After storage (+4°C)
Pre-culture	6.14	4.80	4.53	4.33
CP milk alternative	6.76			
CP media	6.58			

Table B.2. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 2nd trial

	0 h	4 h	6 h	7h	8h	After storage (+4℃)
Pre-culture	6.31	5.66	5.16	4.9	4.75	4.41
CP milk alternative	6.67					
CP media	6.54					

Table B.3. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 3rd trial

	0h	4h	5h	5.5h	After storage (+4℃)
Pre-culture	6.05	5.25	4.9	4.8	4.31
CP milk alternative	6.57				
CP media	6.53				

Table B.4. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 4th trial

	0h	5h	5.5h	After storage (+4℃)
Pre-culture	6.12	5.04	4.81	4.48
CP milk alternative	6.70			
CP media	6.57			

Table B.5. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 5th trial

	0h	4.5h	After storage (+4℃)
Pre-culture	6.02	4.62	4.42
CP milk alternative	6.71		
CP media	6.60		

Table B.6. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 6th trial

	0h	4h	5.5h	After storage (+4℃)
Pre-culture	6.14	5.49	4.85	4.61
CP milk alternative	6.58			
CP media	6.46			

Table B.7. pH values of CP pre-culture using for the inoculation of plant-based milk alternatives in the 7th trial

	0h	5h	After storage (+4℃)
Pre-culture	6.27	4.58	4.49
CP milk alternative	6.70		
CP media	6.60		

Table B.8. pH values of CP pre-cultures using for the inoculation of PBMAs during the fermentations

	Milk alternative	CP media	0h	4h	4.5h	5h	After storage (+4℃)
Pre-culture 1	6.62	6.47	6.02	5.12	5.01	4.82	4.56
Pre-culture 2	6.59	6.47	6.04	5.10	5.0	4.75	4.42

Table B.9. pH values of the ingredients and F. PBMAs during the fermentation mixed with salad dressings for both replications

	0 h	2 h	5 h	5.5 h	After storage (+4℃)
PBMA	6.64				
	6.55				
F. PBMA1	6.40	6.19	4.93	4.78	4.75
	6.46	6.27	5.60	5.48	5.49

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Table B.9 (cont.)

F. PBMA2	6.45	5.62	4.78	4.72	4.72
	6.39	5.82	5.15	5.05	5.11
F. PBMA3	6.39	6.02	4.84	4.61	4.73
	6.36	5.49	5.12	5.08	5.13
Bty 8b		4.08			
		4.10			
Bty 69		4.19			
		4.21			

Table B.10. pH values of mayonnaise, its ingredients and salad dressings during the shelf life for both replications

	1 st day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
AQF	6.12					
	5.95					
Vinegar	3.10					
	2.92					
Mayonnaise	4.04	4.02	4.03	4.0	3.98	4.01
	3.94	3.95	3.97	3.93	3.96	3.92
SD 1	4.27	4.32	4.21	4.33	4.28	4.20
	4.37	4.11	4.23	4.26	4.22	4.21
SD 2	4.25	4.19	4.17	4.24	4.15	4.13
	4.27	4.20	4.16	4.20	4.18	4.18
SD 3	4.24	4.16	4.14	4.20	4.14	4.12
	4.29	4.19	4.15	4.19	4.18	4.19

APPENDIX C. Results of Microbial Analysis

Table C.1. LAB count in the seventh trial of F. PBMAs

	10^{-8}	10^{-7}	10^{-6}
Pre-culture	3	36.5	309
F. PBMA1	2.5	8	129
F. PBMA2	0.5	12.5	117.5
F. PBMA3	2	18	145.5
F. PBMA4	2	9.5	137.5

Results present the mean value of two parallel (n = 1)

Table C.2. LAB count for salad dressing with formulation 1 and with formulation 2 for day 1

	10^{-7}	10^{-6}	10^{-5}
		Day 1	
salad dressing with formulation 1	6.5	51	472
salad dressing with formulation 2	6	54	469
		Day 5	
salad dressing with formulation 1	10.5	83	774
salad dressing with formulation 2	8	63.5	630

Results present the mean value of two parallel (n = 1)

Table C.3. McFarland, pH, and optical density (OD) values of LAB in the fermentations for two replications

	Bty 73	**Cty 44**	**Bty 8b**	**Bty69**
McFarland	8.6	7.4	8.5	8.5
	8.4	8.2	8.9	8.5
pH	4.25	5.47	4.08	4.19
	4.36	5.43	4.10	4.21
OD (at 600nm)	0.521	0.248	0.482	0.428
	0.465	0.283	0.481	0.471

Table C.4. The results of total viable count (PCA) for salad dressing

PCA (10^5 CFU/mL)	SD1	SD2	SD3
1st day	30.5	35	39
1st week	58.5	61	84
2nd week	88	94	84
3rd week	143.5	137.5	134.5
4th week	152.5	153.5	180
5th week	102	138	225
10th week	46.5	100	111.5

Results present the mean value of two parallel for two replications (n =2)

Table C.5. LAB count for used bacteria in the fermentations for two replications

Bacteria	10^{-8}	10^{-7}	10^{-6}
Bty 73	4.25	39.5	324
Cty 44	3.25	23.25	240
Bty 8b	1.5	14.75	109.5
Bty 69	1	7	58.75

Results present the mean value of two parallel for two replications (n =2)

Table C.6. LAB count for CP pre-culture and F. PBMAs using MRS and M17 agars

	10^{-8}	10^{-7}	10^{-6}
		MRS	
Pre-culture	1.5	26.75	237.5
F. PBMA1	1.75	13.75	125.75
F. PBMA2	1.75	14.5	126
F. PBMA3	2.25	17.25	154.75
		M17	
Pre-culture	3	16	159.5

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Table C.6 (cont.)

F. PBMA1	3	10.75	105.75
F. PBMA2	11	11.5	106.25
F. PBMA3	1.25	14	129.75

Results present the mean value of two parallel for two replications (n =2)

Table C.7. LAB count for salad dressing using MRS and M17 agars

		Bacterial count (10^7 CFU/mL)					
		Preriod of storage					
Media	**Salad dressing**	**1. day**	**7. day**	**14. day**	**21. day**	**28. day**	**35. day**
MRS	**SD 1**	5.75	6.25	9.75	8.5	12.5	18.5
	SD 2	2.5	13	15.25	12.75	13.25	17
	SD 3	7.75	10.5	12.5	17	22	22.25
M17	**SD 1**	4	7.5	13.25	12.25	12	16.25
	SD 2	5	7.5	11.25	12.75	16.5	21.5
	SD 3	7	10.25	15	13.75	15.5	22.25

Results present the mean value of two parallel for two replications (n =2)

APPENDIX D. Brix and Titratable Acidity of Mayonnaise, SDs, PBMA, and FPBMAs

Table D.1. Brix value of samples

	1st day	**1st week**	**3rd week**	**5th week**
Mayonnaise	16.9±3.82	16.8±0.71	17.7±3.68	18.65±3.18
SD 1	6.4±2.26	5.5±1.56	6.6±1.41	6.8±1.70
SD 2	6.75±2.47	5.5±1.70	8±0.0	7.4±0.28
SD 3	6.6±2.26	7.3±1.84	8.05±0.49	7.65±1.34
PBMA	2.2±0.0			
F. PBMA1	0.45±0.21			
F. PBMA2	0.75±0.35			

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Table D.1 (cont.)

F. PBMA3	0.35±0.07

Results present the mean value ± standard deviation (n =2)

Table D.2. Titratable acidity of mayonnaise and SDs

	1st day	**1week**	**3week**	**5week**
Mayonnaise	3.21±0.31	3.20±0.82	3.20±0.41	2.77±0.21
SD 1	3.8±0.2	3.79±0.41	3.79±0.0	4.08±0.0
SD 2	3.50±0.0	3.50±0.0	3.50±0.0	4.37±0.41
SD 3	4.02±0.0	3.93±0.21	3.79±0.0	3.64±0.21

Results present the mean value ± standard deviation (n =2). Results were expressed as % acetic acid.

APPENDIX E. Mineral Content of SDs, Mayonnaise, and PBMA

Table E.1. Mineral content of SDs, mayonnaise, and PBMA

	PBMA	**Mayonnaise**	**SD1**	**SD2**	**SD3**
Ca (mg/kg)	1448.168	154.119	112.526	131.518	33.027
Cu (mg/kg)	0.282	34.533	1.361	2.007	0.715
Fe (mg/kg)	ND	ND	ND	ND	ND
K (mg/kg)	376.057	356.456	377.148	403.540	405.686
Mg (mg/kg)	192.125	58.021	58.788	63.929	64.028
Mn (mg/kg)	ND	896.230	65.676	155.194	78.865
Na(mg/kg)	135.086	1047.201	513.243	618.259	560.461
P(mg/kg)	231.439	109.585	135.014	200.613	155.751
Zn (mg/kg)	43.243	34.609	8.369	77.393	2.267

APPENDIX F. Trolox Standard Graphic for ABTS Assay

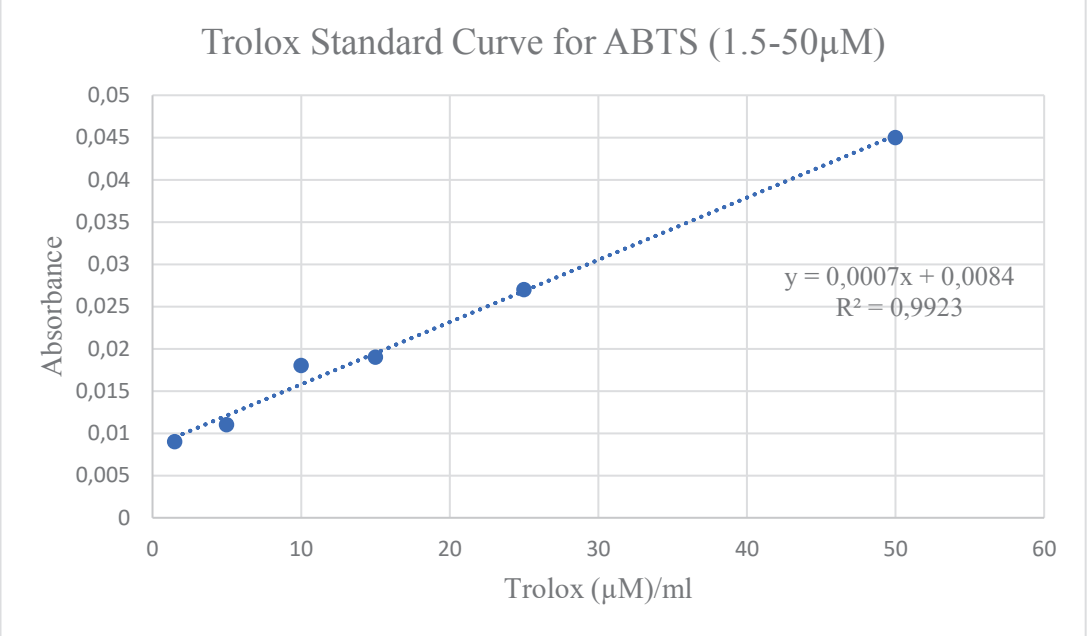

Figure F.1. Trolox standard graphic for ABTS assay

APPENDIX G. L-Leucine Standard Graphic for OPA Assay

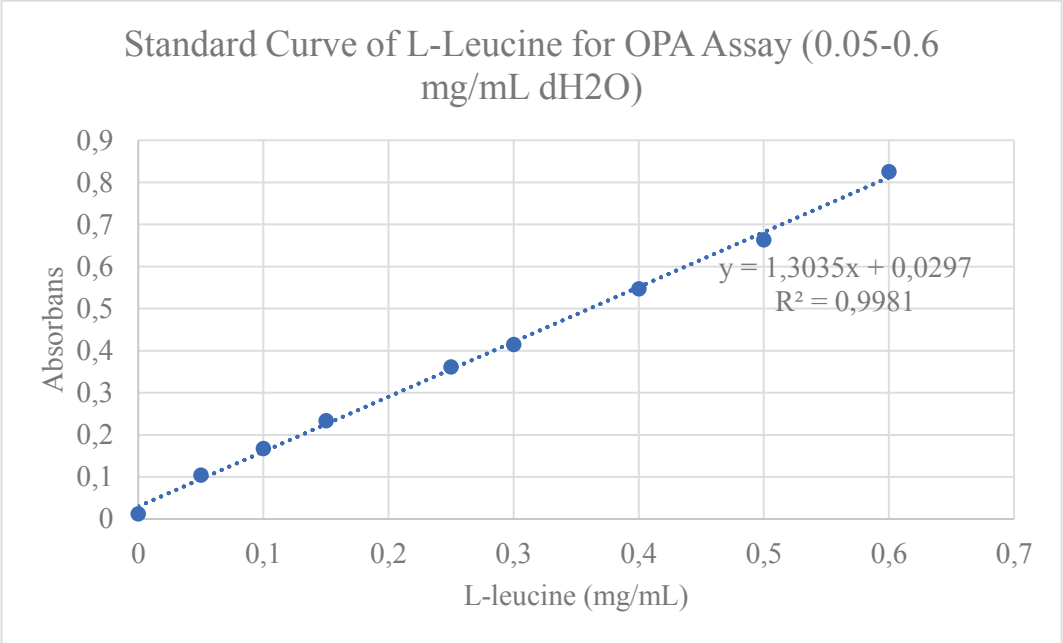

Figure G.1. L-Leucine standard graphic for OPA assay

APPENDIX H. Standard Curve of HA and The Results of ACE-I Activities of Samples

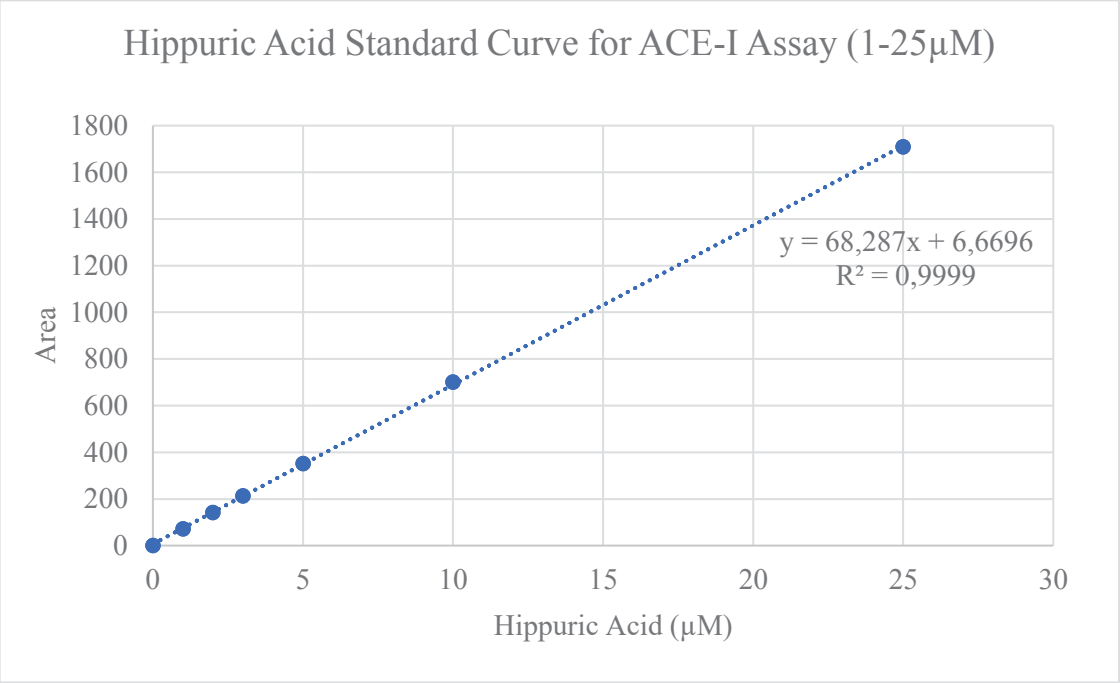

Figure H.1. Hippuric acid (HA) standard curve for ACE-I assay

Table H.1. The results of ACE-I analysis (%) of salad dressings during the shelf life

Time	SD1	SD2	SD3
1st week	82.54±0.01^{Aa}	93.56±0.01^{Ab}	93.98±0.01^{Ab}
3rd week	94.79±0.04^{Ba}	94.97±0.00^{ABa}	95.04±0.02^{ABa}
5th week	95.57±0.01^{Ba}	96.00±0.01^{Ba}	96.27±0.07^{Ba}

[a]Results were expressed as mean ± standard deviation from 2 measurements and 2 replications (n = 2).

[b]The different uppercase letters in the same column mean that the samples are significantly different at different storage times. The different lowercase letters in the same row mean a significant difference between samples (α=0.05).

Table H.2. The results of ACE-I analysis (%) of PBMA, F. PBMAs, and mayonnaise

	ACE-I (%)
PBMA	38.87
F.PBMA1	94.69 ± 0.0^{A}
F.PBMA2	92.89 ± 0.04^{AB}
F.PBMA3	91.11 ± 0.01^{B}
Mayonnaise	90.14

[a]Results were expressed as mean ± standard deviation from 2 measurements and 2 replications (n = 2). Results were expressed as mean values for mayonnaise and PBMA (n = 1).

[b]The different uppercase letters in the same column mean a significant difference between samples (α=0.05).