OPTIMIZATION AND EVALUATION OF THE PROCESS PARAMETERS OF INDIGENOUS CUCUMBER FERMENTATION

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ABSTRACT

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The problems such as microbial load, high cost for process, unwanted final product, organoleptic problems can be encountered during natural and additive free fermentation process in industrially production. The aim of this study to develop an industrially producible and acceptable process without heat treatment for cucumber pickle products. Natural herbal extracts (thyme, dill, bay leaf, rosemary, cinnamon, basil) were applied on cucumber pickle samples. Total of six herbal extracts were used in pickle samples at different concentrations from 50 ppm to 2000 ppm. Statistical analyses were given 3 best extracts to optimize physical and sensorial properties at the end of first design. Physical, chemical, organoleptic, and shelf-life processes of pickles were observed via Stat Ease®. Statical analysis were completed with One Factor at a Time in Design Expert. Box Behnken Design was conducted for 3 herbal extracts (dill, bay leaf, and thyme) to decide optimum scale of extracts. It is decided that dill and bay leaf extracts using process could be an equivalent for industrially production instead of thermal treatments of canned foods. In this context, this thesis was supported by 2 different studies in the period from 2019 to 2022. These studies were about naturally fermentation process and carbon footprint analysis of canned products. These studies form a basis for this thesis.

Keywords: Herbal extract, natural fermentation, cucumber pickle, design expert, One Factor at a Time, Box Behnken.

ÖZET

DOĞAL SALATALIK FERMANTASYONUNUN PROSES PARAMETRELERİNİN OPTİMİZASYONU VE DEĞERLENDİRİLMESİ

Gül, Hazal

Biyomühendislik Yüksek Lisans Programı

Tez Danışmanı: Dr. Öğr. Üyesi Mine Güngörmüşler

Temmuz, 2022

Endüstriyel üretimde doğal ve katkısız fermantasyon işlemi sırasında mikrobiyal yük, yüksek işlem maliyeti, istenmeyen nihai ürün, organoleptik problemler gibi sorunlarla karşılaşılabilmektedir. Bu çalışmanın amacı, salatalık turşusu ürünü için ısıl işlem uygulanmadan endüstriyel olarak üretilebilir ve kabul edilebilir bir proses geliştirmektir. Salatalık turşusu örneklerine doğal bitki özleri (kekik, dereotu, defne yaprağı, biberiye, zencefil, fesleğen) uygulanarak turşuların fiziksel, kimyasal, organoleptik ve raf ömrü süreçleri Stat Ease® ile gözlemlenmiştir. One Factor at a Time ve Box Behnken methodları ile deneysel tasarım kullanılarak istatistiksel analizler tamamlanmıştır. Turşu örneklemelerinde 50 ppm ile 2000 ppm arasında farklı konsantrasyonlarda 6 adet bitki ekstraktı kullanılmıştır. Fiziksel ve duyusal özellikleri optimize etmek için istatistiksel analizlere göre en iyi 3 ekstrakta karar verilmiştir. Optimum ekstrakt ölçeğine karar vermek için 3 bitkisel ekstrakt (dereotu, kekik ve defne yaprağı) arasında Box Behnken Design methodu uygulanmıştır. Konserve gıdaların ısıl işlemleri yerine dereotu ve defne yaprağı ekstraktının kullanılmasının endüstriyel üretim için muadil bir proses ile uygulanabileceğine karar verilmiştir. Çalışma kapsamında 2019-2022 döneminde 2 farklı çalışma ile bu tez desteklenmiştir. Bu çalışmalar konserve ürünlerin doğal fermantasyon süreci ve karbon ayak izi analizi ile ilgili olup bu tezin temelini oluşturmaktadır.

Anahtar Kelimeler: Bitkisel ekstrakt, doğal fermentasyon, salatalık turşusu, deneysel tasarım, One Factor at a Time, Box Behnken.

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CHAPTER 1: INTRODUCTION

1.1. Fermentation Process

The fermentation process has been used for various purposes such as providing protection and stability in foods from past to present. Fermentation is a preservation and storage method, and it is one of the ancient methods to canned foods. Many of foods can ferment such as meat, dairy production, fruits, vegetables, etc. and at the end of fermentation, many of end product and bacteria could be produced with different types of fermentation. Aerobic and anaerobic microorganisms produce energy by electron transport phosphorylation. However, in fermentation process, carbon dioxides, nitrates, and sulfates are used as electron acceptor by denitrifiers or methanogens in lack of oxygen and energy synthesizes by substrate level phosphorylation. Some of external factors affect the type and by/ end products of fermentation. So, the bioprocess continues depends on cultivation method, substrate, metabolism of microorganism, and downstream process (Ray, and Joshi, 2014). This process is a controlled activity of enzymes and microorganisms to alter the physical properties of vegetable, to preserve nutritional value and against decomposition, to enrich, and to give characteristic odour and flavour (Chojnacka, 2010). Different brine and fermentation methods are used in pickle making regardless of home-made or industrial production from past to present.

1.2. Some Preservation Methods in Canned Food Industry and Relevant Reviews

Various pickling methods that frequently utilized in pickle making often provide the preservation and the stability of the vegetable and include acidity regulators, stabilizers, and preservatives. Among the most encountered problems in the industrial canned food industry are CO_2 accumulation in the can (bombing), packages that do not completely cut off the contact of the food with the air, microbial / chemical/ enzymatic reactions in the packaging that continue to lead to degradation, and the textural properties of the raw material cannot be kept at the desired level (Cemeroğlu and Soyer, 2010). These degradations are reflected in industrial production as waste and loss of raw materials. Although heat treatments are mostly used in the canned food industry in our country to extend the storage times and prevent spoilage, these processes cause high energy consumption in the process (Singh et al., 2019). At the end of 2020, input-output analysis of pickle production was carried out in each production process with real-time production data as an example of energy consumption during production. The extensive use of natural gas, electricity, and steam resources were observed in heat treatment process as a result of the environmental life cycle assessment (LCA) study (Gül et al., 2021). Heat treatment methods apply in different ways to preserve foods such as pasteurization, sterilization, baking, roasting, frying, etc. Pasteurization, one of these methods, is generally used in canned product industry to extend shelf life and reduce microbial viability with the help of heat effect on molecules (Aamir et al., 2013). Thermal processes reduce enzyme activity with high temperatures by denatured their structure and end viability of undesirable microorganism like pathogens, yeasts, non-spore forming strains, and moulds (Safefood 360, 2014).

Due to the increasing interest of canned food industry in Turkey and in the world, it is seen that the utilization of water, steam, and heat in industrial production cause natural resource consumption. This study aims at reducing the depletion of resources depending on use of energy and water with new generation trends. Different extracts whose antimicrobial, antifungal and antibacterial properties have been supported by academic studies which aims to protect by using herbal extracts as an alternative method to products whose shelf life and stabilization is provided by heat treatments and is preserved. While determining the extracts to be used, the most common problems encountered in the period since the pickle production were considered, taking into account the main sources of deterioration. One of the most common factors in pickle production is the softening factor caused by pectolytic and cellulolytic enzymes (Voldrich et al., 2009). As seen in Figure 1, the breakdown of glucose to pyruvic acid by microorganisms via glycolysis pathway is commonly utilized (Prasirtsak et al., 2019). Followed by the most common fermentation (trace) pathway of glucose, Embden-Meyerhof-Parnas trace pathway. After that, different products are formed according to the enzymes present in the microorganism (Kim et al., 2020). Lactic acid formation is expected during fermentation as proof of lactic acid bacteria and can be seen in metabolic pathway (Fig. 1).

In food industry, fermentation is the catabolic pathway which partially oxidized compounds like carbohydrates with the help of releasing energy in lack of electron acceptor. At the end of reaction, final electron acceptors would produce from breakdown of carbohydrates as organic compounds. Fermentation also ensures increasing the nutritional value of the food with the essential amino acid synthesis and vitamins during bioprocess. Lactic acid fermentation can conclude the production of lactate and it is depending on the pathway used for glucose oxidation this pathway is called homolactic fermentation. The pathway which results other end products like lactate, acetate, and CO^2 is called heterolactic fermentation. ATP is produced by phosphorylation at the substrate level, and it is main product of fermentation. The energy amount of fermentation is related to initial substrate and pathway of product and also recovery of energy is low to compare aerobic respiration (Forster, and Gescher, 2014).

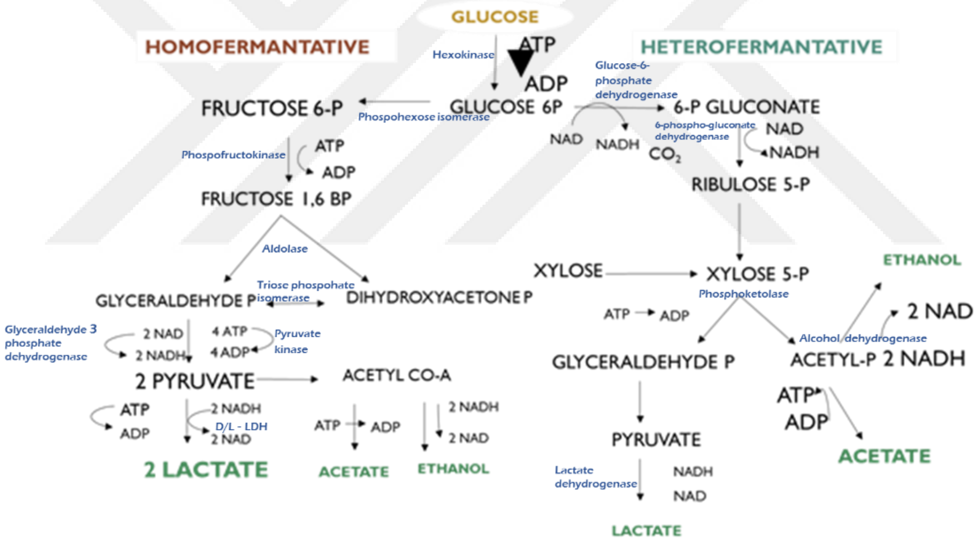

Figure 1. Metabolic fermentation pathways of glucose and final product formations by lactic acid bacteria. Blue codes represent key enzymes of pathway, green codes represent final products (Source: Novik et al., 2017; Prasirtsak et al., 2019; Sabra et al., 2011).

The protection of fresh pickles has been provided with fermentation for increasing food security since from time immemorial. Despite industrial production, homemade pickled vegetables that are the products of spontaneous lactic acid fermentation are widely produced for household consumption. Generally, different types of vegetables are compressed in a vessel and soaked in a vinegar-acidified brine solution prepared based on its organoleptic properties to make pickles. Reducing pH is one of the hurdle effects for canned foods. The variation of microbial population is directly related to salt concentration and temperature profile during fermentation and storage. In general Lactobacillus, Leuconostoc, Weissella, Enterococcus, and Pediococcus are the common lactic acid bacteria (LAB) genera isolated from raw or spontaneously fermented vegetables and fruits (Swain et al., 2014).

The cell cannot show normal activity at low pH and tries to remove excess proton and rebalance the intracellular pH via H+-ATPase (Fig. 2). In the meantime, a high level of energy is spent and acid anions accumulate in the cell. Prolonged exposure of the cell to organic acid causes its death as a result of energy deficiency and anion accumulation.

Lower pH is safe for protection of canned and pickled products (Code of Federal Regulation, 1999).

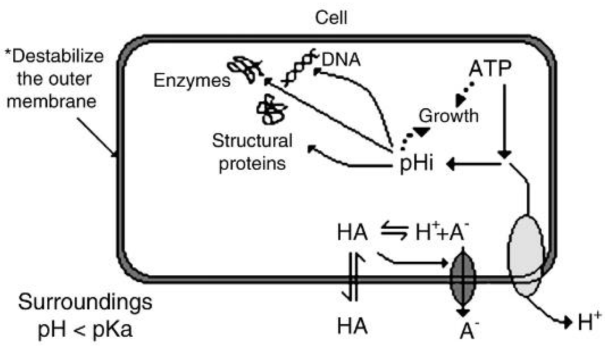

Figure 2. Organic acid mechanism for antimicrobial effects of cell (Mani-Lopez et al., 2012).

The temperature being above 30^{o}C during fermentation is an important quality factor affecting the pickle quality and raw material structure (Behera et al., 2020). Advantages such as less energy consumption to prevent situations such as the fermentation temperature being above room temperature, the continuation of the enzyme effect originating from the raw material, the lactic acid bacteria not using the nutrients in the raw material and the uncontrollable growth of pathogens, and to avoid problems such as unwanted yeast, mold, softening, and bad odor during production. Multiple obstacles technologies (hurdle technology) can be used during to prevent pathogen growth. The reason for this to optimize many factors instead of adjusting a single environmental parameter to extreme growth conditions (Montville et al., 2012). It is an integrated perspective that deals with all methods known as developed methods only against mold/ yeast growth and development in foods when it was first used. One of the first hurdle technology used in history is thought to be leavening, which was discovered by chance. Accordingly, in 7000 BC, the Harappan Civilization in India discovered that yeasts provided preservation and thus prolongation of storage time, while keeping the products in wooden barrels during the making of fermented beverages such as beer (Acar-Tek, and Kocaadam, 2016). The developed method of self-preservation by fermentation has survived to the present day as a lacto-fermentation method since the earliest times of history as seen previous studies about fermentation technology. It is aimed to preserve raw materials by using variable fermentation methods to stabilize the raw materials and extend their shelf life. In order to protect food and to use it for a long time, barrier technologies are developed against enzymes and microorganisms that cause spoilage, generally considering the parameters of pH, temperature, moisture activity and protein structure (Behera et al., 2020).

Although industrial pickle production is mostly done by the brine method, the rate of pickles produced without heat treatment is very slow. Although home-type naturally fermented pickles are produced and consumed frequently in the Turkish society, they are not produced in an industrial scale. The possible health effects of fermented pickles, their reduced salt content, and their suitability for daily consumption increase their preferability (Iqbal, 2014). Considering the health benefits of foods with natural ingredients, the added value of these product increases. Funcitonal foods are natural foods that are formed specifically one or more functions like health effect, easily accessible or transportation. These type of foods modify with some of functional properties (Tur, and Bibiloni, 2016). With the popularization of functional foods, the demand for these products has increased for research in this area. Currently, the methods used in the pickle industry include stabilization by adding $CaCl_2$ and Ca^+ containing compounds to the brine at appropriate concentrations, use of preservatives and pasteurization (Uylaşer, and Başoğlu, 2016). Depending on the use of $CaCl_2$ during the fermentation of pickled cucumbers, it has been determined that certain salts can be used as barrier technology in the process and delay spoilage, based on the structure tests (Akbaş, and Şahin, 2001). Various studies in the pickling industry show effects of desalting methods with calcium salts. One of them was made by Buescher and Burgin (1998), the firmness was examined and they observed that pre-salting methodology with calcium and alum support the structure of pickle and improve against softening (Buescher, and Burgin, 1988).

The use of chemical preservatives such as sodium benzoate, potassium sorbate or propionic acid salts are performed in comparison with pasteurization with high temperature to provide low cost and stabilization of final product (Yi et al., 2020). It has been reported that the spores produced by yeasts develop in the presence of oxygen and increase the humidity of the environment, thereby facilitating the development of pathogens by creating a suitable moist environment for pathogenic bacteria (Alegbeleye et al., 2018). The main ones are; Torulaspora are species such as Hansenula, Candida, and Torulopsis. To inhibit *L. monocytogenes*, *S. aureus*, *C. sakazakii*, *E. coli*, *S. typhi*, *P. aeruginosa*, as well as yeasts, benzoic acid and their salts are commonly used in industry.

Another methodology that is discovered during natural fermentation is preservation with bacteriocins or inhibitors. Bacteriocins are formed by bacterial ribosomes and are discharged extracellularly as polypeptide molecules with antimicrobial activity against exiguous associated or broad-range microorganisms. They are formed by lactic acid bacteria that accepted as Generally Regarded as Safe (GRAS) by the US Food and Drug Administration (FDA). Bacteriocins are popular as a content of food technology because of their proved effects on pathogens that caused food spoilage. They have received increasing attention in the industry as natural antimicrobials and bio preservatives due to customer request for fresh taste and slightly protected foodstuffs. Traditional fermented fish, vegetables, dairy products, and meat are common sources of bacteriocin-forming LAB. These bacteriocins have been demonstrated to inhibit the expansion of foodborne pathogens including *Salmonella*, *L. monocytogenes, and S. aureus*. (Pang et al., 2022). Pathogens that develop depending on the raw material quality and oxygen availability can inhibit with lactic acid bacteria and their bacteriocins. Pathogens, as well as yeasts that occur in contact with oxygen and support pathogen growth in the environment, inhibited by lactic acid bacteria and their bacteriocins during fermentation. Beyond these, bacteriocins are not effective as thermal treatments during providing shelf life of canned foods because of commercial sterilization processes in industry.

Microbial safety and textural properties of canned foods could be provided with the help of thermal or some other treatments like water activity impact, gamma, UV, electromagnetic field, pressure applications, microfiltration, etc. (Putnik et al., 2020). Table 1 shows some hurdle effect trials applied in food industry. On the other hand, the formation of antimicrobial/antibacterial properties against pathogens during microbial growth is also a preservation method for several food products including fermented vegetables. These types of methods that avail for preservation are called hurdle technology. Although there are some hurdles to ensure safety of food, structural and flavour properties should also be considered while deciding on the technology.

Table 1. Some hurdle technology trials on pathogens in food or beverage products.

Methodology	**Results**	**Pathogens or yeasts**	**Reference**
Lactobacillus plantarum and *Lactobacillus paracasei* were provided from cows from a local farm and the obtained LAB were stored until used in MRS culture. Ultrasonication at low wavelength (37 kHz, 160 W) was used to heal Bovine colostrum and its effect was observed in different combinations. Free and bounded fatty acid analysis were made and comparisons were made.	Ultrasonication, fermentation, and dehydration have been shown to inhibit microbial contamination and growth. No pathogen was found in samples that contain LAB.	*E.coli*, Enterobacter, mold, and yeast.	(Bartkiene et al., 2018)

Table 1 (continued). Some hurdle technology trials on pathogens in food or beverage products.

The application was continued by sonication with 20 kHz, 750 W power. As an example, UHT milk and Saline solution (SSS) were used and microbial decline was observed via transmission electron microscopy. The aim of the study is to observe the efficiency of the power ultrasound system on selected microbes.	A significant reduction in microorganisms was observed in selected samples (SSS and UHT).	*Escherichia coli*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*	(Cameron et al., 2008)
Using UHT milk, orange and carrot juice samples, the application was made by providing ultrasound and thermal stability at 20 kHz and 50, 110 or 160 µm power, and the growth of gram positive and gram-negative bacteria was observed.	The growth of gram positive and negative bacteria was inhibited, and their numbers decreased.	*Escherichia coli* and *Lactobacillus acidophilus*	(Zenker et al., 2003)
Taste, odor, structure, chemical and microbiological examinations were made on skimmed and semi-skimmed milk by pasteurization and	1st and 7th day analyses were taken from milk samples kept in cold air. A decrease in growth was	Aerobic, mesophilic bacteria, total coliform bacteria, *Staphylococcus spp.*,	(Balthazar et al., 2019)

ultrasound experiments in different combinations. Ultrasound trials were completed at 20 kHz and 130 W power at different time intervals.	observed in log term. Gram negative species were not found while Lactobacilli were found in the same trials.	Lactobacilli, and *Lactic streptococci*	

Thermal methods are common and conclusive processes for food preservation and quality. Pasteurization is the safest method for fresh pack type pickles in terms of low and high thermal treatment ability. Yavuz et al. (2018) focused on an alternative for tunnel type pasteurization and pickle qualifications after heat implementations. In industrial pickle production, at that microorganism growth threat risk, softening enzymes like pectinase also join in the critical control points. To prevent softening, shrinkage, pathogen population, and yeast reproduction; pasteurization temperature and other parameters should be well defined for hurdle technology (Yavuz et al., 2018). Although there are several thermal treatment methods used in industry including pasteurization, sterilization, oven drying, microwave, irradiation; heat treatment of end product, not all of them are effective. Only pasteurization and sterilization methods are used in industrial hurdle technology for packaged goods. While sterilization is generally conducted for dairy products, pasteurization is used for canned, bottled, or boxed foods and beverages (Khan et al., 2017).

Various studies aiming to stabilize the end product by using different extracts in order to prevent the development of bacteria and yeast strains and to prevent softening and putrefaction by ensuring quality are included in the literature. Results such as oxidation control, reduction of metal ions and prevention of undesired lipid peroxidation have been noted due to the use of rosemary and sage (Baştürk, and Tabar, 2018). On the other hand, high antioxidant activity was observed in oil and oil emulsions due to the use of thyme (Abdalla, and Roozen, 1999). Herbal extracts contain different amounts and types of p-hydroxy benzoic acid, gallic acid, ferulic acids, caffeic acid, ellagic acid, quercetin, vanillin, p-coumaric, etc. They prevent the formation of some yeasts, molds and pathogens by showing antimicrobial and antioxidant effects with the help of phenolic compounds (Baştürk, and Tabar, 2018). These include rosemary (Salvia rosmarinus), *S. aureus*, *E. coli* and *L. monocytogenes* used in the meat storage and production industry and proven to inhibit *E. coli*, *S. enteritidis*, *L. monocytogenes* bacteria (Soyer et al., 2020). Yellow mustard (*Sinapis alba*) which has been shown to have antimicrobial properties due to the benzoic acid it contains, as a result of studies on its effectiveness against yeast, thyme (*Thymus vulgaris*), which is reported to be effective against pathogens in fermented meat and dairy products, aromatic and it contains volatile components and at the same time, its antimicrobial and antibacterial properties with the aldehydes in its structure have been determined as a result of in vitro studies and it has been proven to have high antibacterial properties against *K. pneumoniae*, *B. megaterium*, *S. aureus*, *E. coli*, *E. cloacae* and S. *faecalis* (Turgis et al., 2009; Soyer et al., 2020; Laranjo et al., 2019). Cinnamon (*Cinnamomum verum*), basil whose effect against yeast has not been proven, but whose inhibition feature against bacteria and microbes has been carried out (*Ocimum basilicum*), with flavonoid compounds in its content during fermentation, which plays an auxiliary role in fermentation and with phenolic compounds and known antimicrobial effect, vine leaves (*Vitis*), mint extract and lavender oil for up to 20 days when used together (Nabavi et al., 2015; Suppakul et al., 2003; Amin Jaradat et al., 2017). Lemon leaf (*Citrus limon*), which acts as a natural preservative and contains strong flavonoids on its own, olive leaf, which contains strong phenolic compounds and has been found to have inhibitory properties against pathogenic bacteria and yeasts, which is a strong antioxidant with its high amount of oleuropein in its structure (Viji et al., 2015). Olive leaf extract (*Olea europaea L.*), and lastly bay leaf (*Laurus nobilis*), it is frequently used in the canning industry as a flavoring and has various antioxidant effects (Licciardello et al., 2015).

There are dill (*Anethum graveolens*), thyme (*Thymus vulgaris*), rosemary (*Salvia rosmarinus*), bayleaf (*Laurus nobilis*), cinnamon (*Cinnamomum verum*), and basil (*Ocimum basilicum*) extracts, which are used in industries to provide storage and shelf life (Yılmaz et al., 2013).

Anethum graveolens L. is called as dill in society. It has a significant aromatic taste for tradition Mediterranean cuisine especially foods, sauces, herbs and pickles. It is affluent in terms of minerals such as magnesium, phosphorus, and potassium. Because of effective usage, easy transportation, and shelf life of dill, dried leaves are preferable in industry. Aromatic composition of dill leaves was examined and mostly limonene, linoleic acid, dill adipole, and calvone were present (İşbilir, and Sağıroğlu, 2011; Singh et al., 2019). The antimicrobial activity of *Anethum graveolens* were detected and over 100 µg of dried extract/ ml in dill extracts were observed in DPPH assay (Shyu et al., 2009).

Thymus vulgaris is commonly known as thyme and its essential oil generally use as antimicrobial on different lines such as cosmetics, food products, and drugs. Iseppi et al. (2019) were observed antimicrobial activity of thyme and rosemary on ready to eat vegetables. They found minimum inhibitory concentration as between 4 and 8 µg / ml separately. However, they decided that the best result of antibacterial effect consists of with combination of two essential oils. They observed that combination of herbal essences reduces the bacterial load of ready to eat vegetables and protect throughout shelf life (Iseppi et al., 2019). In another research about *Thymus vulgaris'* antimicrobial and antibacterial effect was conducted against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Main phenolic compound is observed as tannic acid in dry matter at the end of content determination. They mentioned about thyme as a natural antioxidant that easily accessible for food products and drugs (Nadia, and Rachid, 2013).

Salvia rosmarinus is commonly named as rosemary. Health effect and antioxidant activity of rosemary were observed by Veenstra and Johnson (2021). They interpreted that rosemary extract was rich in terms of carnosol (CL), rosmarinic acid (RA), and carnosic acid (CA). High carnosol intake provide to protect shelf life on food products (Veenstra, and Johnson, 2021). So, rosemary extract has suppressor effect against food pathogens especially modified atmosphere packaged products as above sample.

Laurus Nobilis is known as bay leaf has therapeutic properties like antimicrobial, antibacterial, and anti-inflammatory. The sensitive structure of polyphenols has led to the seeking an alternative for directly usage on target. Thus, encapsulation methodology was tried for bay leaf with stabilisers. Microcapsulation method has provided to protect the presence of gallic acid until 50% that gained antioxidant properties to herbs. So, the therapeutic properties of herb were protected and it would easily apply on food products as preservative (Chaumun et al., 2020).

Cinnamomum verum with the common name cinnamon has antioxidant role on food and drug industry. In 2022, it was used as preservative during stabilizing shelf life of fish product. Even features of meat and vegetable products are so different to each other, it is measurable research to determination of pathogen inhibition. *Cinnamonum verum* extract has high amount of gallic acid at the end of scavenging radicals DPPH method. To conclude cinnamon has regressive effect on lipid oxidation. Thus, reducing lipid oxidation provides antioxidant effect and finally protect shelf life until 6 months (Simbine et al., 2022).

Ocimum basilicum that is known as basil also has some therapeutic effects on food, drug, and feed industry. Pathogen inhibitory effect of extract was experimented by Takwa et al. (2018) on bread samples. Some Gram positive and negative bacteria were used to control. Bread brewed with *Ocimum basilicum* had best result, the extract affected more effective than E codes food preservatives like potassium salts (Takwa et al., 2018).

This thesis is focused on herbal extracts and their inhibitory effects on the shelf life of cucumber pickles. Table 2 reports the minimum and maximum values used as additives for the most used extracts. Two studies which done by the author and the supervisor constitute the fundament of this study (Gül et al., 2021; Gül, and Güngörmüşler, 2022).

Table 2. Literature review for various amounts of herbal extracts in different implementations.

Extracts	Min. concentration	Max. concentration	Referances
***Olea europaea L.* (mg/kg)**	0 mg/kg	4000 mg/kg	(Testa et al., 2019)
***Laurus Nobilis* (mg/kg)**	Limit is not detected	Limit is not detected	
***Citrus limon* (mg/kg)**	0 mg/kg	200 mg/kg	(Viji et al., 2015)
***Vitis* (mg/kg)**	0 mg/kg	5000 mg/kg	(Hamzawy et al., 2013)
***Ocimum basilicum* (mg/kg)**	Limit is not detected	Limit is not detected	(Takwa et al., 2018)
***Cinnamomum verum* (mg/kg)**	0 mg/kg	450 mg/ kg	(Vidanagamage et al., 2016)
Thymus vulgaris	Limit is not reported	Limit is not reported	-
***Salvia rosmarinus* (mg/kg)**	0 mg/kg	200 mg/kg	(Veenstra, and Johnson et al., 2021)
***Anethum graveolens* (mg/kg)**	0 mg/kg	150000 mg/kg	(Shyu et al., 2009)

CHAPTER 2: METHODOLOGY

Fresh cucumbers from Odemis, Menemen, Adana, Maras, and Antalya for naturally fermented pickle were kindly provided from Euro Gıda Corporate, Izmir, Turkey. To examine the effects of the extracts individually and multiple to each other, the Stat-Ease Design Expert program is used with the response surface method. Box-Behnken experimental design followed by validation trials as a result of first OFAT optimization. Trials will be conducted with the 3 most significant extracts given in Table 3 in terms of single effect and interaction with each other. According to the determined amounts, concentration values will replace the -1 - +1 values in Table 4 at the end of study. Chemical and sensorial analysis are conducted at the end of whole design processes to observe fermentation process and commercial sterilization. Commercial sterilization is defined with safety pH value in canned foods such as in industrial area the difference of pH should not be more than 0.5 during incubation period (McGlynn, 2016). The brine which added different amount of extracts was prepared with defined concentrations as created by a OFAT (Design Expert software (version 10.0.7.0, Stat-Ease Inc., Minneapolis, USA) (Montgomery et al., 2012)) (Table 3) were added to fresh, raw and clean cucumbers in 720 ml glass jars. 6-9 cm caliber, and a total amount of 370-380 g raw cucumbers were cleaned by washing in tap water and were mixed with the brined and placed into the jars in sterile conditions to prevent microbial contamination. Cucumbers were picked from local territory in Izmir district, thus, accordingly, slight differences in size were observed. A burning flame was used around the jars to ensure cleaning. The cucumbers were naturally fermented with the indigenous flora of the vegetables at different herbal extract concentrations for 7 days.

2.1. Chemical Analysis

2.1.1 pH measurement

For pH analysis on the final product, 10 ml samples taken from the pickle sample brine was tested in duplicate using a digital pH meter (Mettler Toledo, Switzerland) by immersion method.

2.1.2 Acidity analysis

Acid analysis was done by titration method, and 0.1 mL of brine was used with 0.6 ml of phenolphthalein. The titration was carried out with 0.1 N sodium hydroxide until a pink color was observed. The amount of spent sodium hydroxide was observed in the burette and multiplied by the acidity coefficient to reach the product % acidity.

2.1.3. Salt analysis

For salt analysis, 0.6 ml potassium chromate was used as an indicator, and 0.1 N silver nitrate was consumed by titration with silver nitrate until a red-brown color was observed. The observed amount was multiplied by the salt coefficient and the % salt in the product.

Equation: ((Consumption of 0,1 N $AgNO_3$ in titration (mL)) * (Normality of $AgNO_3$) * (Factor of 0,1 N $AgNO_3$) * 0.585 * 100) / mass of sample (g) (Sezey and Adun, 2019)

2.2. Microbial analysis (Total bacteria count)

Viable counts of microorganisms were carried out by pour plate method in 1 mL samples taken from brine in products whose incubation is completed in 0.1% (W/V) peptone water (pH= 7.2 ± 0.2). PCA (Plate Count Agar) agar was used for total mesophilic aerobic bacteria counts and incubation will take 3-4 days at 30° C. In 48 hours (2 days), yeasts and molds were began to observed.

Colony count of the organisms will be made and reported as cfu/ml.

2.3. Sensory analyses

It was planned to ask the panelists 6 different questions on 7 scales: color, smell, sourness, sweetness, product taste and general product acceptability. As a control, the Melis brand cucumber pickle from Euro Gıda will be tasted by the panelists. In order to test whether there are statistically significant differences between the groups while evaluating the results, reporting was out in the SigmaPlot v14 Software using the ANOVA method, with a 95% confidence interval.

The target values are for the cucumber pickle in industrial manufacturing, the pH value is at least 4.0; sensorial is at least 5 (I like it a little); storage period is at least 1 year; acidity value is at least 0.5% (W/V).

2.4. Statistical Experimental Designs

The concentrations of the extracts mentioned within the scope of the thesis were prepared at 3 levels with One Factor at a Time (Table 3). A factorial design from the experimental design methods were used to test whether the commercial sterilization can be met. Another experimental design study was carried out with the best 3 extracts that is determined as a result of these trials, and the most appropriate concentrations for the longest storage are determined by Box Behnken Response Surface Methodology.

Table 3. One Factor at a Time Design matrix.

Run	Factor 1 A: Thyme mg/L	Factor 2 B: Dill mg/L	Factor 3 C: Bay leaf mg/L	Factor 4 D: Rosmarin mg/L	Factor 5 E: Cinnamon mg/L	Factor 6 F: Basil mg/L
1	2000.00	1250.00	500.00	125.00	281.00	500.00
2	1250.00	2000.00	1250.00	125.00	112.00	2000.00
3	500.00	1250.00	1250.00	50.00	112.00	1250.00
4	1250.00	500.00	2000.00	125.00	112.00	1250.00
5	500.00	2000.00	1250.00	200.00	281.00	1250.00
6	2000.00	2000.00	1250.00	50.00	281.00	1250.00
7	1250.00	1250.00	500.00	50.00	281.00	500.00
8	1250.00	500.00	500.00	125.00	112.00	1250.00
9	500.00	1250.00	1250.00	200.00	112.00	1250.00
10	1250.00	1250.00	1250.00	125.00	281.00	1250.00
11	1250.00	1250.00	2000.00	50.00	281.00	500.00
12	1250.00	500.00	1250.00	125.00	450.00	2000.00
13	500.00	500.00	1250.00	200.00	281.00	1250.00
14	1250.00	1250.00	500.00	200.00	281.00	2000.00
15	1250.00	1250.00	500.00	50.00	281.00	2000.00
16	500.00	1250.00	500.00	125.00	281.00	2000.00
17	1250.00	2000.00	1250.00	125.00	112.00	500.00
18	1250.00	1250.00	2000.00	200.00	281.00	2000.00
19	1250.00	1250.00	500.00	200.00	281.00	500.00
20	1250.00	1250.00	1250.00	125.00	281.00	1250.00
21	500.00	1250.00	2000.00	125.00	281.00	2000.00
22	2000.00	1250.00	500.00	125.00	281.00	2000.00
23	2000.00	2000.00	1250.00	200.00	281.00	1250.00
24	1250.00	1250.00	1250.00	125.00	281.00	1250.00
25	1250.00	1250.00	1250.00	125.00	281.00	1250.00
26	2000.00	500.00	1250.00	200.00	281.00	1250.00
27	1250.00	500.00	1250.00	125.00	450.00	500.00

Table 3 (continued). One Factor at a Time Design matrix.

28	1250.00	1250.00	1250.00	125.00	281.00	1250.00
29	1250.00	2000.00	500.00	125.00	450.00	1250.00
30	2000.00	1250.00	1250.00	50.00	450.00	1250.00
31	500.00	2000.00	1250.00	50.00	281.00	1250.00
32	1250.00	2000.00	2000.00	125.00	450.00	1250.00
33	1250.00	2000.00	1250.00	125.00	450.00	500.00
34	1250.00	500.00	1250.00	125.00	112.00	2000.00
35	2000.00	500.00	1250.00	50.00	281.00	1250.00
36	2000.00	1250.00	2000.00	125.00	281.00	2000.00
37	1250.00	2000.00	500.00	125.00	112.00	1250.00
38	1250.00	2000.00	1250.00	125.00	450.00	2000.00
39	500.00	500.00	1250.00	50.00	281.00	1250.00
40	2000.00	1250.00	1250.00	50.00	112.00	1250.00
41	1250.00	2000.00	2000.00	125.00	112.00	1250.00
42	1250.00	1250.00	2000.00	200.00	281.00	500.00
43	1250.00	500.00	2000.00	125.00	450.00	1250.00
44	2000.00	1250.00	1250.00	200.00	112.00	1250.00
45	1250.00	500.00	1250.00	125.00	112.00	500.00
46	500.00	1250.00	1250.00	200.00	450.00	1250.00
47	500.00	1250.00	2000.00	125.00	281.00	500.00
48	1250.00	1250.00	2000.00	50.00	281.00	2000.00
49	1250.00	500.00	500.00	125.00	450.00	1250.00
50	500.00	1250.00	500.00	125.00	281.00	500.00
51	500.00	1250.00	1250.00	50.00	450.00	1250.00
52	1250.00	1250.00	1250.00	125.00	281.00	1250.00
53	2000.00	1250.00	1250.00	200.00	450.00	1250.00
54	2000.00	1250.00	2000.00	125.00	281.00	500.00

According to the optimization study obtained as a result of the one factor at a time design, in order to observe the stabilization and final product quality in the products obtained, total bacteria count, texture and titratable acidity amount analysis including microbiological, physical and chemical analyses mentioned in the final product analysis section was made. Following a Box-Behnken experimental design was applied with independent and dependent variables; factors and response parameters; -

1, 0, and 1 are contain the lowest, medium, and highest concentration (ppm) values, respectively. Table 4 was used to optimize the amount of extract.

The statistical design of experiments, regression analysis of the data, and model building were performed via the Design-Expert software (version 10.0.7.0, Stat-Ease Inc., Minneapolis, USA) (Montgomery et al., 2012). The experiments were developed with three factors, three levels, 17 runs, using a Box-Behnken Design (BBD) Response Surface Methodology (RSM), including the central point (five replicates). The independent parameters, also referred as factors, were herbal extract contents, each at three coded levels − 1, 0, 1 as shown in Table 4.

Table 4. Box Behnken Design matrix (Design Expert v12).

Run	**Factor 1: Dill (ppm)**	**Factor 2: Bay leaf (ppm)**	**Factor 3: Thyme (ppm)**
1	1250	1250	1250
2	500	500	1250
3	1250	2000	500
4	500	1250	2000
5	1250	500	2000
6	2000	500	1250
7	500	2000	1250
8	1250	1250	1250
9	1250	1250	1250
10	500	1250	500
11	1250	1250	1250
12	1250	500	500
13	2000	2000	1250
14	1250	2000	2000
15	1250	1250	1250
16	2000	1250	500
17	2000	1250	2000

Figure 3. Cucumber pickle beginning of fermentation and end of incubation time.

CHAPTER 3: RESULTS & DISCUSSION

The fermented pickle products were successfully produced during the experiments. The pH difference is 0.5 or above in 18 out of 32 samples applied to the extract as a result of the 7 days incubation period as seen in Table 5. For a product to be considered commercially sterile, the commercial sterilization pH must be below 0.5. As seen from the experiments, the samples used at the highest rates of extract yielded commercial sterile results in general. After the acid, salt and final product analyses of the remaining 14 samples were made, they were tasted by Euro Gıda employees. The participants stated that the "extract taste is very intense" in general and determined the average of their general opinion as 2 (I did not like it) in Table 5. Only 3 extract taste that commercially sterilise and sensorial 5 level (I like it a little) were considered in Box-Behnken Design and they show green highlight in Table 6. Tasters preferred dill, thyme, and bay leaf tastes in final product.

Table 5. Radar plot of sensorial analysis results

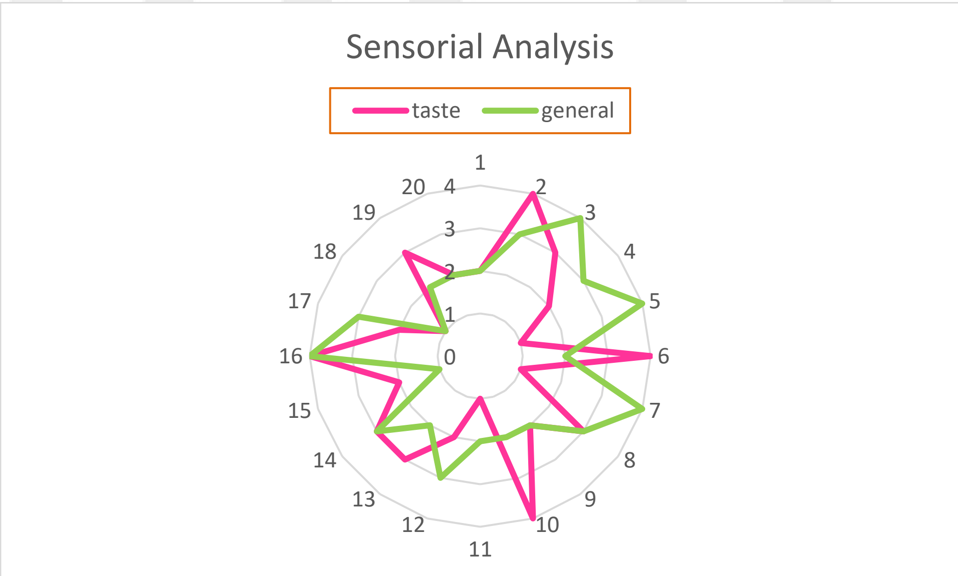

Table 6. Results of 2 factorial design analysis at One Factor at a Time

Response 3: Salt %	1.42	1.56	1.64	**1.62**	1.58	1.49	1.51	1.65
Response 2: Acidity %	0.62	0.58	0.61	**0.52**	0.66	0.71	0.59	0.6
Response 1: Ph 2 at 7th day of fermentation	3.60	3.62	3.24	**3.97**	3.56	3.94	3.90	3.88
Response 1: Ph 1 at beginning of	4.05	4.10	4.12	**4.02**	4.03	4.32	4.02	4.22
Factor 6 F: Basil mg/L	500	2000	500	**2000**	2000	2000	2000	500
Factor 5 E: Cinnamon mg/L	112	112	450	**450**	450	450	112	450
Factor 4 D: Rosemary mg/L	50	50	250	**250**	50	50	250	250
Factor 3 C: Bay leaf mg/L	500	500	500	**500**	2000	2000	500	2000
Factor 2 B: Dill mg/L	2000	2000	500	**2000**	500	2000	2000	500
Factor 1 A: Thyme mg/L	2000	500	500	**500**	2000	500	2000	2000
Run	1	2	3	**4**	5	6	7	8

Table 6 (continued). Results of 2 factorial design analysis at One Factor at a Time

9	2000	500	2000	50	112	500	4.26	3.31	0.55	1.66
10	2000	500	500	250	450	2000	4.03	3.30	0.63	1.5
11	2000	2000	2000	50	450	500	4.11	3.28	0.69	1.39
12	500	500	500	50	450	2000	4.14	3.27	0.57	1.43
13	2000	500	2000	250	112	2000	4.11	2.46	0.51	1.58
14	500	2000	500	50	450	500	4.09	2.22	0.47	1.62
15	500	500	2000	250	112	500	4.56	2.22	0.58	1.6
16	2000	500	500	250	112	500	4.01	2.63	0.73	1.59
17	2000	2000	2000	250	112	500	4.48	3.88	0.7	1.58

18	500	500	500	50	112	500	4.16	2.87	0.68	1.68

Table 6 (continued). Results of 2 factorial design analysis at One Factor at a Time

27	500	500	2000	50	112	2000	4.18	3.69	0.54	1.57
28	500	2000	2000	250	450	500	4.02	3.73	0.59	1.49
29	2000	2000	500	250	450	500	4.32	3.69	0.67	1.5
30	2000	2000	500	50	450	2000	4.75	3.60	0.65	1.49
31	500	500	500	250	112	2000	4.85	3.84	0.71	1.43
32	2000	2000	2000	50	112	2000	4.03	3.76	0.61	1.6
19	2000	500	500	50	112	2000	4.34	2.87	0.61	1.64

Table 6 (continued). Results of 2 factorial design analysis at One Factor at a Time

20	500	2000	500	250	112	500	4.23	2.87	0.66	1.64
21	2000	500	500	50	450	500	4.10	3.60	0.57	1.57
22	500	2000	2000	50	112	500	4.12	3.57	0.55	1.55
23	500	2000	2000	250	112	2000	4.07	3.72	0.56	1.46
24	500	500	2000	250	112	2000	4.17	3.67	0.51	1.48
25	500	500	2000	50	450	500	4.25	3.57	0.59	1.52
26	2000	2000	2000	250	450	2000	4.22	3.46	0.68	1.48

***Most optimal result was given in bold by the software.**

****Not commercial sterilized runs. These trials are ignored in next design (red highlight).**

*****Selected extracts for Box Behnken Design (green highlights).**

As seen in Table 6, commercial sterilization essentiality was not met in red runs because their pH difference is more than 0.5 during incubation time. These runs formed unsuccessful results for perspective of safety food without heat treatment. The analysis in Stat Ease® were conducted for interaction of thyme, dill, and bay leaf extracts combinations to compare effects of extracts.

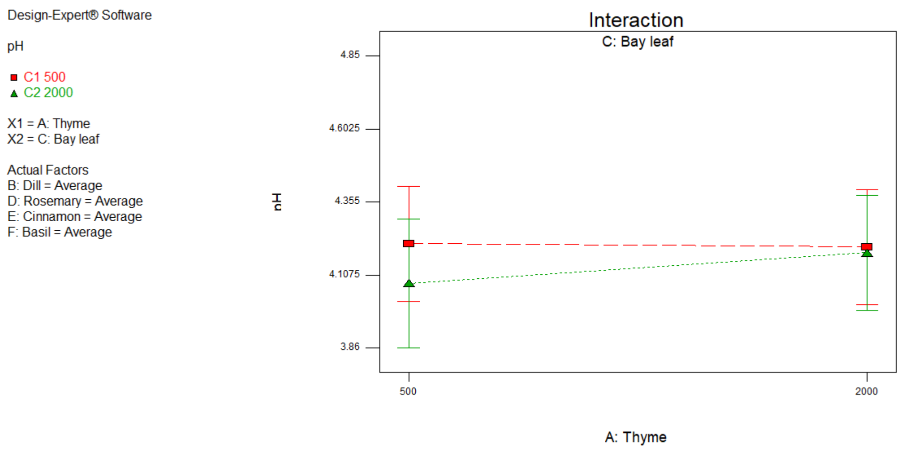

Figure 4. Interaction of bay leaf and thyme on pH results at beginning of fermentation

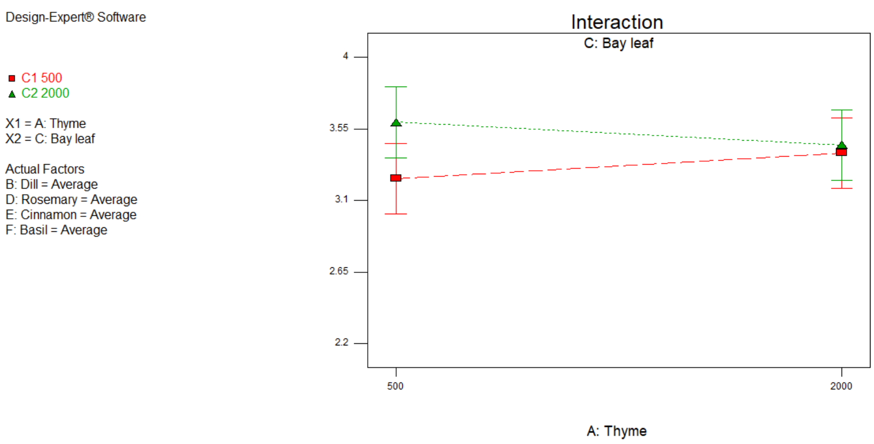

Figure 5. Interaction between bay leaf and thyme at 7th day of fermentation on pH of cucumber pickle samples.

When the extracts are average level in pickle sample except thyme and bay leaf pH levels were not regularly decrease or increase either maximum or minimum amounts of extract. However, pH values were significantly decrease during incubation period as seen in Figure 4 and Figure 5.

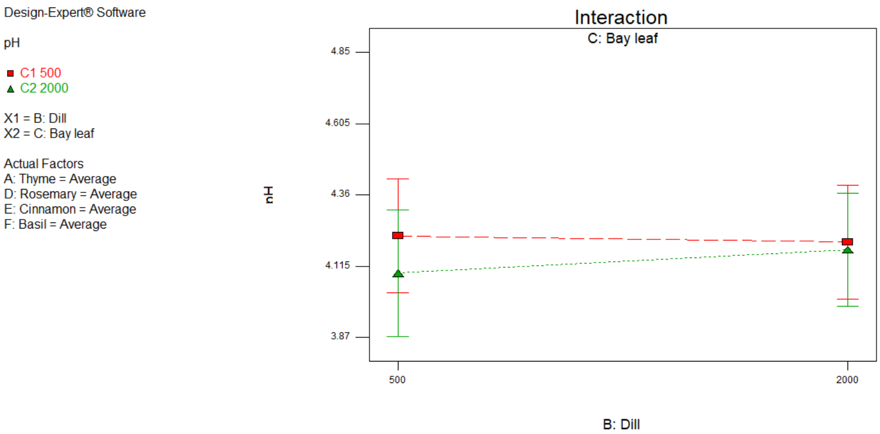

Figure 6. Interaction of bay leaf and dill on pH results at beginning of fermentation.

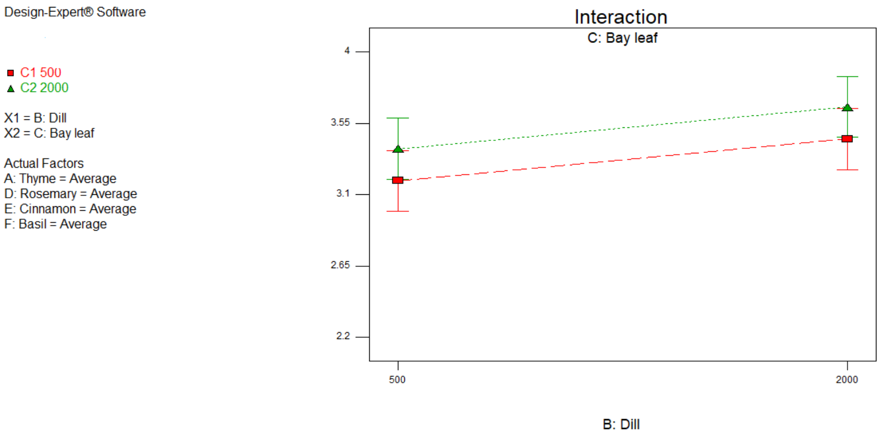

Figure 7. Interaction of bay leaf and dill on pH results and at the end of incubation period (7th day).

The interaction between bay leaf and dill was more significant than interaction with thyme because of linearization at the end of fermentation as seen in Figure 6. While moving on minimum to maximum extract presence caused reducing pH level at beginning of fermentation (Figure 5), it caused increasing pH level at the end of fermentation. Thus, the difference between beginning pH and final pH was less. So, the interaction could be more meaningful for commercial sterilization.

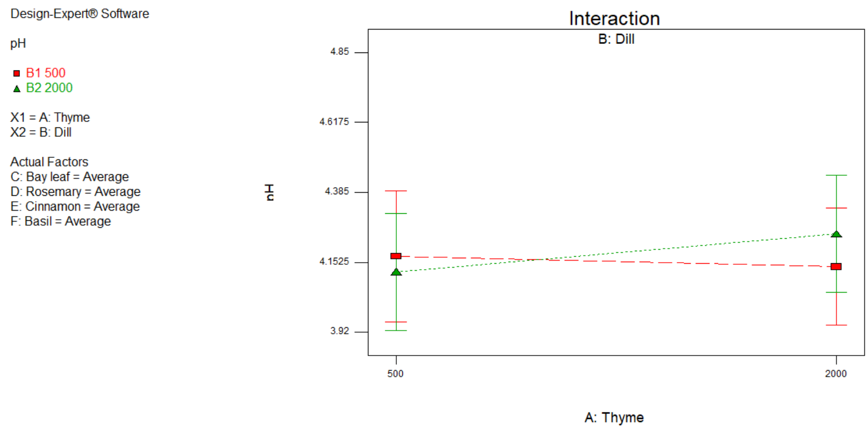

Figure 8. Interaction of thyme and dill on pH results at beginning of fermentation.

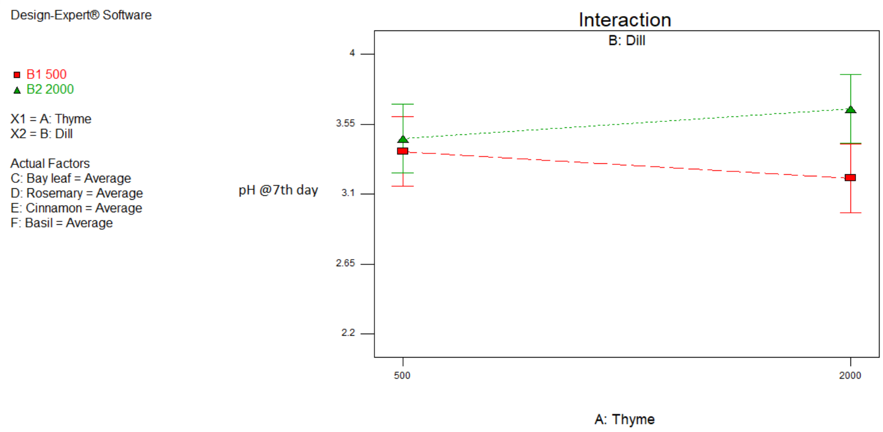

Figure 9. Interaction of thyme and dill on pH results at 7th day of fermentation.

Figure 9 shows the last double interaction between dill and thyme and Figure 5 shows when inverse effect between pH and extract amount. Although there was an inverse effect at the beginning of fermentation, this situation did not proceed at the end of incubation period.

Figure 10 shows triple interaction for thyme, dill, and bay leaf at beginning of fermentation and at the end of incubation period.

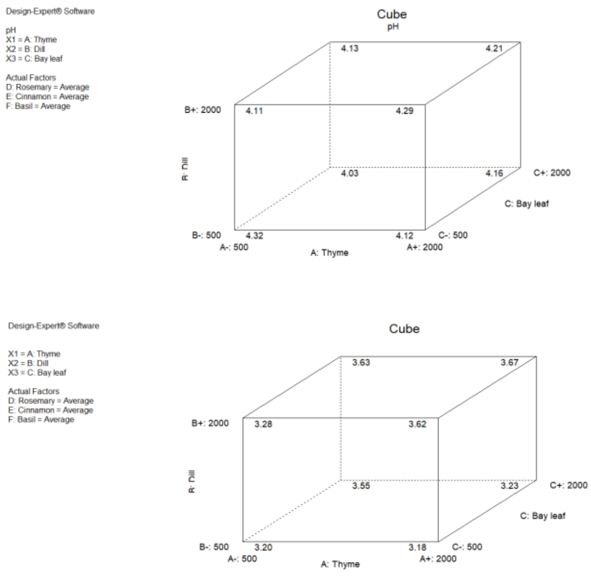

Figure 10. Triple interaction between bay leaf, thyme, and dill for pH alteration.

Figure 10 shows the points of pH during triple interactions when applied maximum and minimum concentrations. Range of 7.1 and 7.3 pH level was observable and significant at beginning, when 3.3 and 3.6 range was significant at 7th day.

The other response when deciding the most appropriate extracts was acidity level. The target was about 0.5% (W/V) titratable acidity. However, 0.6% (W/V) acidity was more significant in terms of analyses as seen in Figure 8.

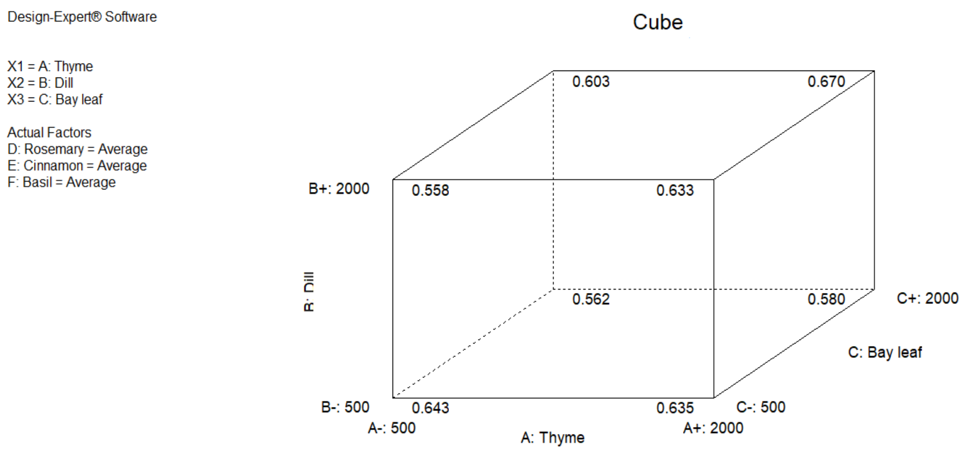

Figure 11. Titratable acidity of pickle products when minimum and maximum amounts of triple interaction.

0.5 – 0.7 % (W/V)

titratable acidity range is acceptable as sensorial and chemically. The acidity level could be changed in terms of presence of LAB and adding acidity regulator as seen in HPLC results of previous study. The result of acidity was significant with usage of extract and also naturally fermentation process (can be seen in following pages). The calculation of acidity during usage of different amounts of extracts is as below.

Equation1: $+0.61 + 0.019 * A + 5.394E^{-}003 * B - 6.644E^{-}003 * C + 0.016 * A * B + 2.269E^{-}003 * A * C + 0.027 * B * C - 4.144E^{-}003 * A * B * C$

A: Thyme, **B**: Dill , **C**: Bay leaf

The unknown titratable acidity percentage of pickle product can be found with the help of Equation 1 when different amount of extracts applied. The other extracts were ignored.

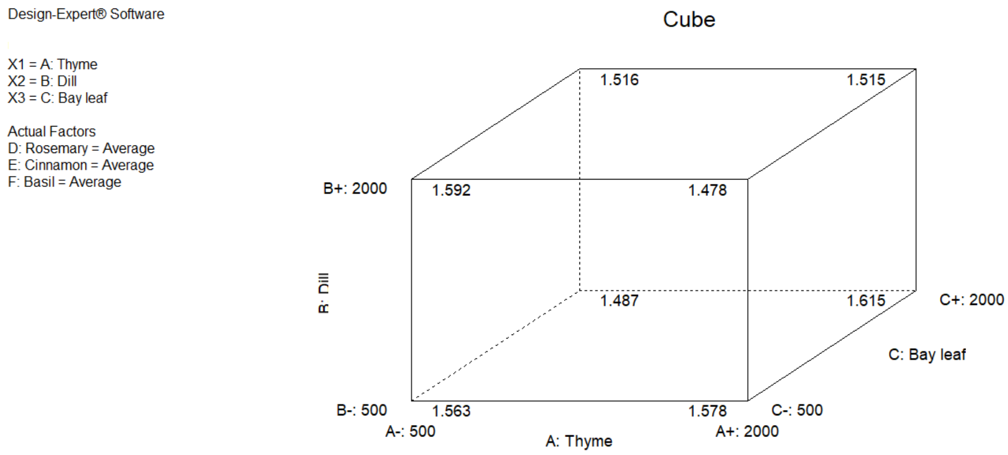

Figure 12. Salt amount of pickle products when minimum and maximum amounts of triple interaction.

The salt concentration was approximately 1,5% (W/V) in industrially production and exposure extract samples. Figure 12 shows titratable salt similarly Figure 11.

Equation 2: $+1.54 + 3.464E^{-}003 * A - 0.018 * B - 9.714E^{-}003 * C - 0.032 * A * B + 0.028 * A * C$

A: Thyme, **B**: Dill , **C**: Bay leaf

The unknown titratable salt percentage of pickle product can be found with the help of Equation 2 when different amount of extracts applied. The other extracts were ignored.

Table 7. Responses of Box Behnken Design.

Run	Factor 1: Dill mg/l	Factor 2: Bay leaf mg/l	Factor 3: Thyme mg/l	Response 1: pH	Response 2: Acidity %	Response 3: Salt %	Response 4: Microbial Load log cfu/ml
1	1250	1250	1250	3.72	0.64	1.57	3.1544
2	500	500	1250	3.67	0.58	1.49	0
3	1250	2000	500	3.57	0.71	1.62	3.8745
4	500	1250	2000	3.46	0.62	1.65	0
5	1250	500	2000	3.69	0.66	1.55	0
6	2000	500	1250	3.73	0.71	1.54	5.2592
7	500	2000	1250	3.69	0.72	1.58	0
8	1250	1250	1250	3.60	0.63	1.57	2.1988
9	1250	1250	1250	3.84	0.62	1.58	0
10	500	1250	500	3.76	0.62	1.53	0
11	1250	1250	1250	3.76	0.65	1.59	4.1234
12	1250	500	500	3.54	0.61	1.6	0
13	2000	2000	1250	3.68	0.73	1.56	0
14	1250	2000	2000	3.39	0.79	1.71	3.9756
15	1250	1250	1250	3.68	0.63	1.66	0
16	2000	1250	500	3.52	0.67	1.59	4.1874
17	2000	1250	2000	3.60	0.7	1.6	0

Table 7 shows the whole response about final product.

Box Behnken Design was used to optimize 3 extract results of cucumber pickle and pH and microbial count were not significant ($p>0.05$); acidity and salt results were significant. It is not expected to be significant due to inconsistent data in microbial load results. Only significant graphs were given in below with previous study results to compare the acidity profile of naturally fermented cucumber pickle and extract added cucumber pickle.

Figure 9 shows the titratable acidity of cucumber pickle at the end of incubation time a, b, and c shows different double interactions.

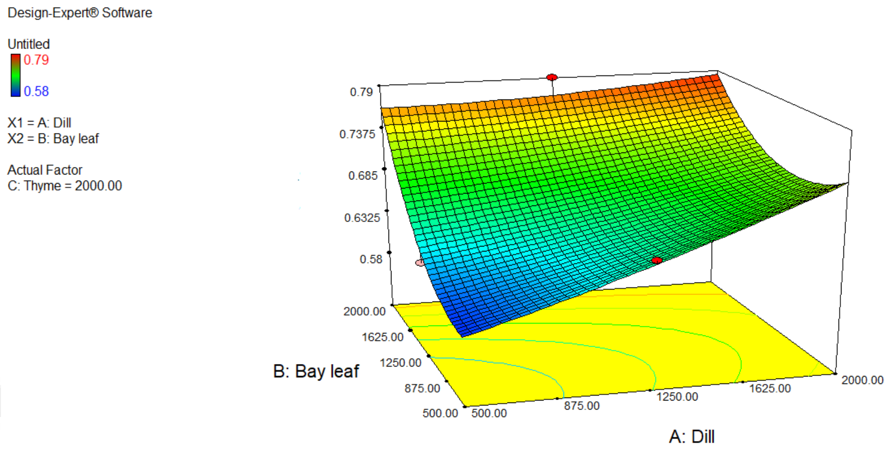

Figure 13. 3D Surface plot of double interaction models on bay leaf and dill on titratable acidity.

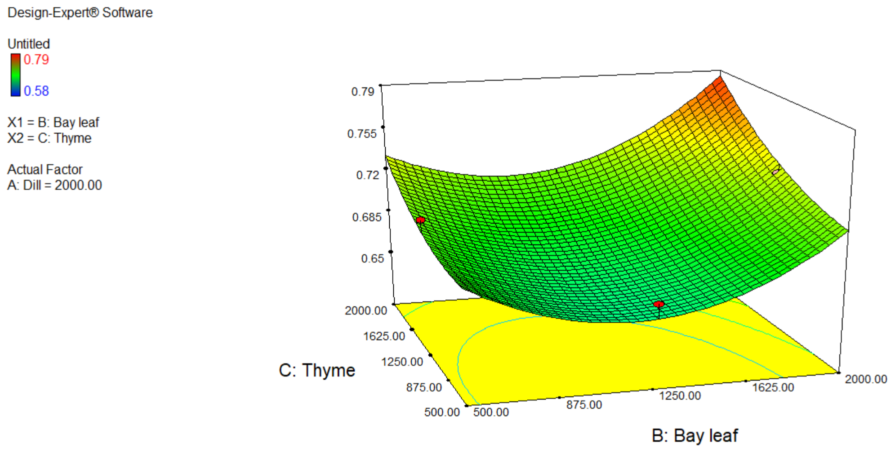

Figure 14. 3D Surface plot of double interaction models on bay leaf and thyme on titratable acidity.

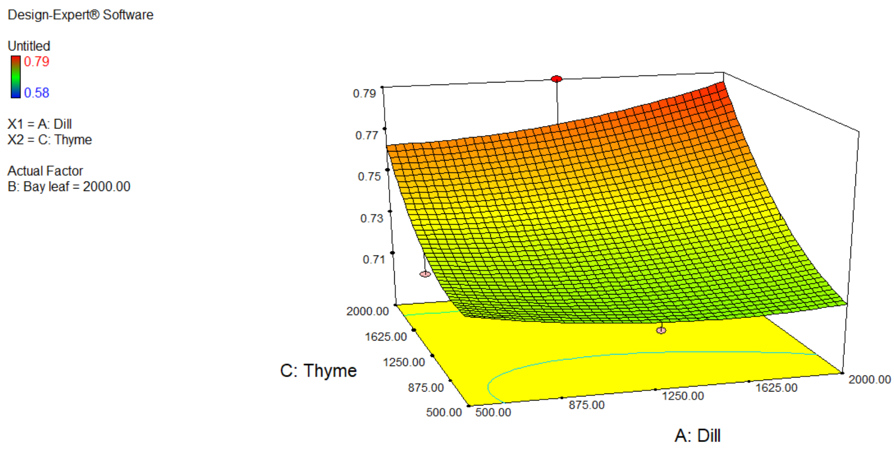

Figure 15. 3D Surface plot of double interaction of bay leaf and thyme on titratable acidity.

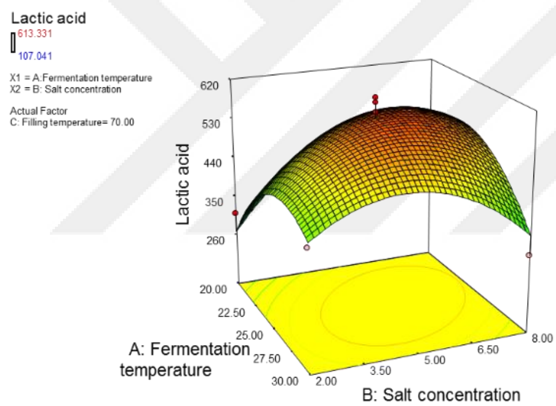

Figure 16. 3D Surface plot of lactic acid (ppm) response with the factors fermentation temperature and salt concentration at the end of 7 days (Source: Gül and Güngörmüşler, 2022).

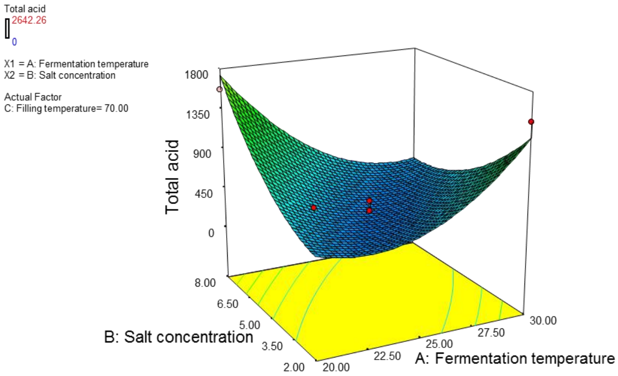

Figure 17. 3D Surface plot of total acid (ppm) response with the factors fermentation temperature and salt concentration at the end of 7 days (Source: Gül and Güngörmüşler, 2022).

When the titratable acidity was approximately 0.6% (W/V) in final product, the lactic acid and total acid amounts were as like Figure 16 and Figure 17 in ppm. On the other hand, 0.6388% (W/V) titratable acidity was optimized value of extract added samples and it is observed mostly in bay leaf and dill interaction as seen in Figure 15.

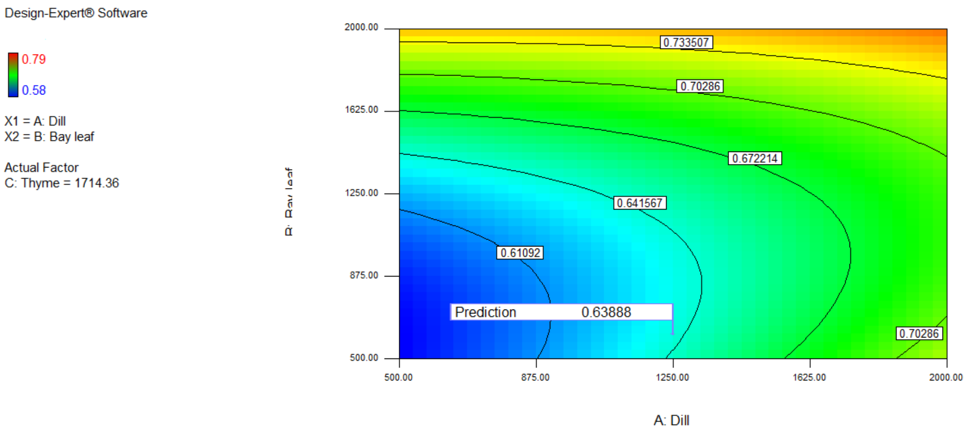

Figure 18. 3D plot of result desirability on bay leaf and dill interaction. At the end of optimization the best value of titratable acidity was shown in bay leaf and dill interaction as 0.6388% (W/V).

Simultaneous optimization of responses is very difficult and complex when assuming too many responses. In this study, the effects of process conditions were investigated in combination with the principal component analysis response surface method, which is an approach that optimizes multiple inputs and outputs simultaneously regardless of number. With this combination, more than one response can be optimized. Figure 18 indicates the results of Box-Behnken Design. Red points on Figures shows optimum conditions in model. If the red points can be applied to second order polynomial equations, the optimum value of response could be estimated.

Maximum amount of total acid of samples is 0.6% (W/V) at the end of the fermentation process. Industrially produced pickles have 0.3-0.8% (W/V) acidity. Accordingly, naturally fermented samples acidity ratio was observed to fit industrial acidity range. Gül and Güngörmüşler were observed glucose amount of naturally fermented pickles in 2021 in same production line when decided to total acidity in cucumber pickle. The results were compare the other studies and given in Table 7.

Table 8. Comparison of glucose (mg/L) and the cumulative amounts of lactic acid and acetic acid (total acid amounts) (mg/L) between different studies and current study. Present study was given in bold.

Raw material	**Initial glucose concentration (mg/L)**	**Total acid concentration (mg/L)**	**pH**	**Reference**
Cucumber	205	482.8	4.0	(Lu et al., 2001)
Cucumber	376.3*	743.7*	-	(McFeeters, 1993)
Cucumber	50	120	<4.0	(Pérez-Díaz et al., 2013)
Cucumber	**680***	**1302.48***	**3.8***	(Gül and Güngörmüşler, 2022)

Table 8 (continued). Comparison of glucose (mg/L) and the cumulative amounts of lactic acid and acetic acid (total acid amounts) (mg/L) between different studies and current study.

Cabbage	50	250	<4.0	(Pérez-Díaz et al., 2013)
Traditional maize	200*	1686*	4.0	(Adesokan et al., 2009)

* Average and calculated values from different samples were taken.

** Pilot production that occurred without raw materials showed high amount of acid production correlated with natural fermentation of the raw material.

The amount of acid decrease until 90% when produced by natural fermentation. The glucose/total acid ratio in this study are similar to other natural fermented cucumber results. On the other hand, different raw materials give different amounts of acid. As seen in Figure 17 and 18, and Table 7; acid production capability is affected by type of raw material too in addition salt ratio, fermentation temperature, and filling temperature.

Glucose, fructose, lactose, glycerol, ethanol, acetic acid, and lactic acid amounts were determined at different fermentation times. Microbial availability and pH fluctuation were also simultaneously observed at fixed intervals. Responses of results were followed on 1st, 7th, 10th, and 14th days. At the beginning and end of fermentation, sugar consumption was reported about 730 ppm, while production of total acid was over 300 ppm. The viability of lactic acid bacteria reached maximum level on 7th day, above 6 log cfu/ml. Therefore, cucumber pickle model for consumption and production of sugar and lactic acid bacteria during fermentation fits to the model, and can be used during different types of processes pickled cucumber (Gül and Güngörmüşler, 2022). On the other hand, this sugar consumption to acid production period differentiates at presence of herbal extracts as given in this study. Acid amount was determined in terms of pH fluctuation and commercial sterilization. Even so, naturally fermented pickles gave us an idea about incubation process and deterioration of canned pickles in industrial production.

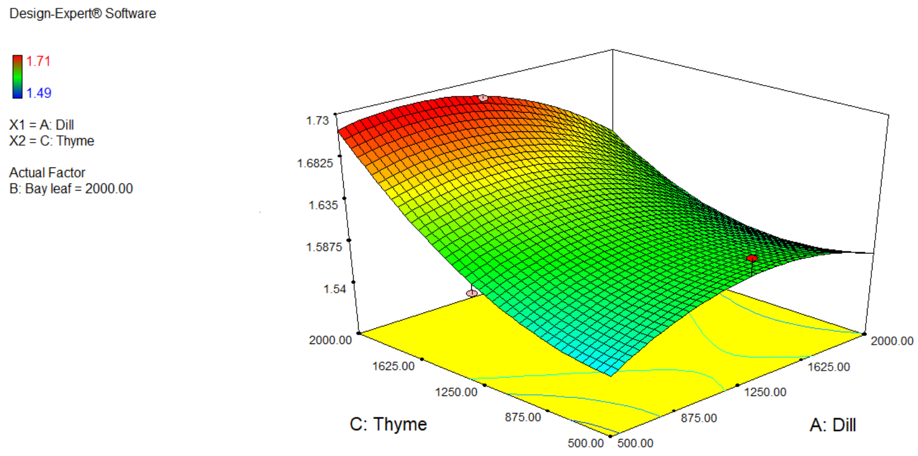

Figure 19. 3D Surface Plots of titratable salt response during double interactions of of thyme and dill extract.

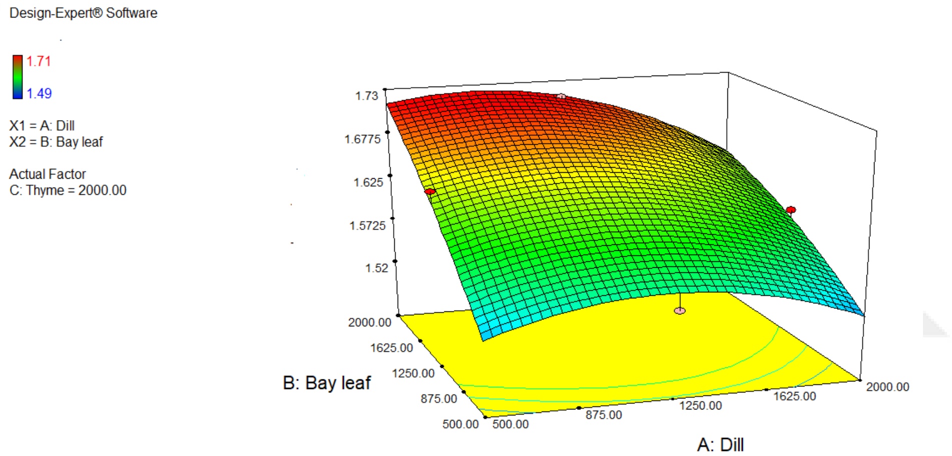

Figure 20. 3D Surface Plots of titratable salt response during double interactions of bay leaf and dill extract.

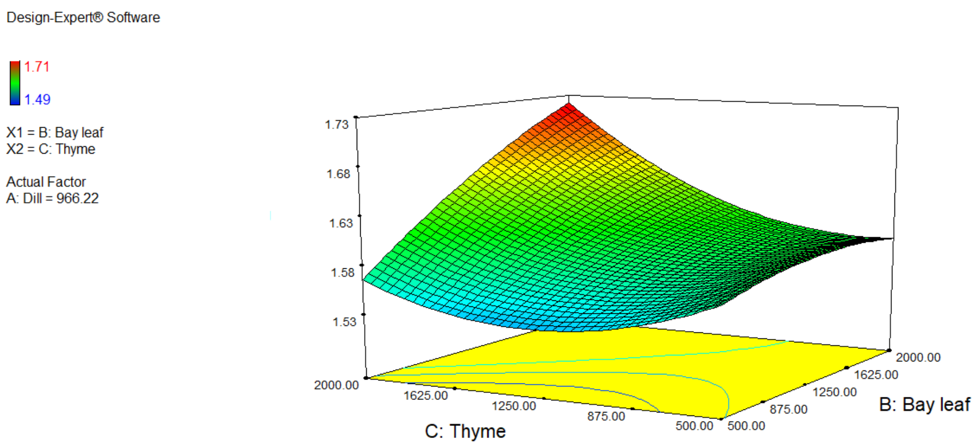

Figure 21. 3D Surface Plots of titratable salt response during double interactions of bay leaf and thyme extract.

Titratable salt results were changed between 1.5-1.7% (W/V) and this range is suitable for industrial production. The prediction of titratable salt result is approximately 1.6% (W/V). This percentage is mostly responded in bay leaf and dill interaction as well as acidity.

Sensorial target value was expected as 5 level "I like it a bit". However, natural paneliststs that provided by Euro Gıda staff evaluated the pickles and they decided 4 level "I uncertain about this".

The incubation period for the canned and pickled groups varies between 7-10 days during production that commercially heat treated. Heat treatments are used to prolong the retention time by preventing the pH decrease due to yeast and mold growth. Depending on the incubation period used in the industry, an idea can be obtained from the first pH measurement whether yeast and mold has formed or not. Thus, it can be observed whether commercial sterilization is achieved or not. Hermetic closures with heat treatments and barriers methods and modified atmosphere packaging can be applied to prevent spoilage in food technologies (Yaralı, 2017). During the production of low acid products in the pickle industry, it is expected that the pH difference should not exceed 0.5 during the product's shelf life when the pH of the products is below 4, together with the lethality of the products that have completed the incubation period and have been pasteurized and stabilized. The BBD responses were collected following the incubation period of the samples. The pickles were kept at room temperature for 7 days, by selecting 3 samples with a pH change of less than 0.5, the correlation between titratable acidity and pH value, and texture closest to the fresh raw material sample formed the basis of BBD optimization. In terms of contributing to the sustainable environment and energy, it is thought that economic gain will be achieved by reducing the use of natural gas and water, with the optimization study without the use of heat treatment. According to the 11^{th} Turkish Republic Development Plan, compliance with the policy of efficient production at low cost is among the desired effects for industrial manufacturers. In this context, this thesis reports that interaction between bay leaf and dill extracts has given most optimal result in terms of microbial load, acidity level, salt level, and sensorial. 500 ppm dill extract, 1250 ppm bay leaf extract, and 500 ml thyme extract is most optimal result for preservation without heat treatment in industrial production process.

CHAPTER 4: CONCLUSION

While deciding on this design, the aim was to develop an industrially producible and acceptable process without heat treatment. In this context, this thesis was supported by 2 different studies in the period from 2019 to 2022. First of all, when heat treatment is applied for 1 jar of pickles, carbon footprint calculations were presented by Gül et al. (2021), all inputs and outputs of the system were analysed by them. As a result of this analysis, it was demonstrated that the use of heat treatment is the part that causes the largest use of natural gas, electricity, and steam in the process. Accordingly, the experiments in this thesis were aimed to provide a protection methodology with the help of the indigenous lactic acid bacteria in naturally fermented products. In this context, all physical and chemical properties were examined by natural fermentation. Although the health effect of the naturally fermentation method is supported, it has been concluded that it cannot be applied without an additional process or additive in industrial scale production. Finally, with this thesis, the effect of herbal extracts applied at different rates on the final product was investigated. Although the studies show that the extracts used have a protective effect on foods, they should be supported by end product analysis. The selected extracts were decided based on the general flavours used in the industry. It has been observed that some extracts have a positive effect on shelf life by inhibiting the growth of pathogenic bacteria in meat and fish products. In another study, it was observed that the extracts applied to the bread product also had an inhibitory effect on the formation of mold and yeast. In this study, it was aimed to observe the effect of herbal extracts in cucumber pickles, based on the effect of inhibiting the growth of bacteria that cause yeast and mold formation. Considering that the extracts will affect not only the shelf life but also the organoleptic profile, the flavours familiar to the industry have been preferred. Instead of investigating the single effect of a single extract, two designs were created to examine the multiple effects of frequently used herbal extracts. The initial design was made to reduce the number of experiments and to optimize the extract concentrations that is added based on the final product analysis. As a result of this design, dill, bay leaf and thyme extracts, which are easier to supply in the industry than others, showed the most positive effect. As a result of the sensorial analysis made for this experimental group, which is expected to be accepted as a taste profile, it was decided that it was acceptable.

When we look at the researches, it is these three herbal extracts that have been examined more than the others.

First of all, OFAT was performed and the three extracts that gave the best results were decided. It was decided that the combination of dill and bay leaf could be used in terms of microbial degradation and sensory by performing the surface response methodology with the use of bay leaf, dill and thyme extracts sensorial and chemically. Although we could not reach the sensory target value of 5, it is foreseen that this study creates a basis that can be used in the food industry, with the support of ensuring the final product subordinate, salt, microbial load and shelf life.

REFERENCES

Aamir, M., Ovissipour, M., Sablani, S.S. and Rasco, B. (2013) *Predicting the quality of pasteurized vegetables using kinetic models: A review*. International Journal of Food Science, 271271, pp. 1-30.

Abdalla, A.E. and Roozen, J.P. (1999) *Effect of plant extracts on the oxidative stability of sunflower oil and emulsion*, Food Chemistry, 64(3), pp. 323–329.

Acar-Tek, N. and Kocaadam, B. (2016) *Ekmek, bira, şarap ve yoğurdun orijinleri ve tarihsel süreçleri*, Beslenme ve Diyet Dergisi, 44(3), pp. 272-278.

Adesokan, I., Odetoyinbo, B. and Okanlawon, M. (2009) *Optimization of lactic acid production by lactic acid bacteria ısolated from some traditional fermented food in Nigeria.* Pakistan Journal of Nutrition, 8(5), pp. 611-615.

Akbaş, H. and Şahin, İ. (2001) *Hıyar turşularında yumuşamanın önlenmesi ve kullanılabilecek kalsiyum klorür miktarının belirlenmesi*, Gıda, 26(5), pp. 333-338.

Alegbeleye, O.O., Singleton, I. and Sant'Ana, A.S. (2018) *Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review*, Food Microbiology, 73, pp. 177–208.

Amin Jaradat, N., Naser Zaid, A., Hussen, F. and Ali, I. (2017) *Asma yapraklarını koruma yöntemlerinin toplam fenol, toplam flavonoit ve antioksidan kapasite üzerine etkileri*, Marmara Pharmaceutical Journal, 21(2), pp. 291–291.

Balthazar, C.F., Santillo, A., Guimarães, J.T., Bevilacqua, A., Corbo, M.R., Caroprese, M., Marino, R., Esmerino, E.A., Silva, M.C., Raices, R.S.L., Freitas, M.Q., Cruz, A.G. and Albenzio, M. (2019) *Ultrasound processing of fresh and frozen semi-skimmed sheep milk and its effects on microbiological and physical-chemical quality*, Ultrasonics Sonochemistry, 51, pp. 241–248.

Bartkiene, E., Bartkevics, V., Ikkere, L.E., Pugajeva, I., Zavistanaviciute, P., Lele, V., Ruzauskas, M., Bernatoniene, J., Jakstas, V., Klupsaite, D., Zadeike, D., Viskelis, P. and Juodeikiene, G. (2018) *The effects of ultrasonication, fermentation with Lactobacillus sp., and dehydration on the chemical composition and microbial contamination of bovine colostrum*, Journal of Dairy Science, 101(8), pp. 6787–6798.

Baştürk, A. and Tabar, B. (2018) *Effects of different herbal extracts on oxidative stability of sunflower oil*, Gıda / The Journal Of Food, 43(2), pp. 333–346.

Behera, S.S., El Sheikha, A.F., Hammami, R. and Kumar, A. (2020) *Traditionally fermented pickles: How the microbial diversity associated with their nutritional and health benefits?*, Journal of Functional Foods, 70.

Buescher, R.W. and Burgin, C. (1988) Effect *of calcium chloride and alum on fermentation, desalting, and firmness retention of cucumber pickles,* Journal of Food Science, 53(1), pp. 296–297.

Cameron, M., McMaster, L.D. and Britz, T.J. (2008) *Electron microscopic analysis of dairy microbes inactivated by ultrasound,* Ultrasonics Sonochemistry, 15(6), pp. 960–964.

Cemeroğlu, B. and Soyer, A. (2005). *Gıdaların Soğutulması ve Dondurulması. Gıda Mühendisliğinde Temel İşlemler Kitabı.* 1st ed. Ankara: Başkent Klişe Matbaacılık, pp.1-356.

Chaumun, M., Goelo, V., Ribeiro, A.M., Rocha, F. and Estevinho, B.N. (2020) *In vitro evaluation of microparticles with Laurus nobilis L. extract prepared by spray-drying for application in food and pharmaceutical products,* Food and Bioproducts Processing, 122, pp. 124–135.

Chojnacka, K. (2010). *Chemical Engineering and Chemical Process Technology-Fermentation Products.* Encyclopedia of Life Support Systems. 5th ed. Wroclaw: EOLSS Publications, pp.189-219.

Code of Federal Regulation. (1999). *Acidified Foods Title 21, Part 114*. Government Printing Office. Washington.

Forster, A.H. and Gescher, J. (2014) *Metabolic engineering of Escherichia coli for production of mixed-acid fermentation end products,* Frontiers in Bioengineering and Biotechnology, 2.

Gül, H. and Güngörmüşler, M. (2022) *Utilizing response surface methodology to evaluate the process parameters of indigenous cucumber fermentation,* Zeitschrift Für Naturforschung C, 0(0).

Gül, H., Üçtuğ, F. G. and Güngörmüşler, M. (2021) *Environmental life cycle assessment of industrially produced pickled and roasted vegetables,* International Journal of Environmental Science and Technology, 19, pp. 8751–8768.

Hamzawy, M., El-Denshary, E., Hassan, N., Mannaa, F. and Abdel-Wahhab, M. (2013) *Dietary supplementation of calendula officinalis counteracts the oxidative stress and liver damage resulted from aflatoxin.* ISRN Nutrition, 2013, pp.1-9.

Iqbal, A. (2014) *Preservation of carrot, green chilli and brinjal by fermentation and pickling*, International Food Research Journal, 21(6), pp. 2405-2412.

Iseppi, R., Sabia, C., de Niederhäusern, S., Pellati, F., Benvenuti, S., Tardugno, R., Bondi, M. and Messi, P. (2019) *Antibacterial activity of Rosmarinus officinalis L. and Thymus vulgaris L. essential oils and their combination against food-borne pathogens and spoilage bacteria in ready-to-eat vegetables*, Natural Product Research, 33(24), pp. 3568–3572.

Khan, M.I., Waqas, M., Hayat, T. and Alsaedi, A. (2017) *A comparative study of Casson fluid with homogeneous-heterogeneous reactions*, Journal of Colloid and Interface Science, 498, pp. 85–90.

Kim, K.H., Chun, B.H., Baek, J.H., Roh, S.W., Lee, S.H. and Jeon, C.O. (2020) *Genomic and metabolic features of Lactobacillus sakei as revealed by its pan-genome and the metatranscriptome of kimchi fermentation*, Food Microbiology, 86, pp. 103341.

Laranjo, M., Fernández-León, A.M., Agulheiro-Santos, A.C., Potes, M.E. and Elias, M. (2019) *Essential oils of aromatic and medicinal plants play a role in food safety*, Journal of Food Processing and Preservation, 46(8).

Licciardello, F., Wittenauer, J., Saengerlaub, S., Reinelt, M. and Stramm, C. (2015) *Rapid assessment of the effectiveness of antioxidant active packaging study with grape pomace and olive leaf extracts*, Food Packaging and Shelf Life, 6, pp. 1–6.

Lu, Z., Fleming, H.P. and McFeeters, R.F. (2001) *Differential glucose and fructose utilization during cucumber juice fermentation*, Journal of Food Science, 66(1), pp. 162–166.

Mani-Lopez, E., Garcia, H.S. and Lopez-Malo, A. (2012) *Organic acids as antimicrobials to control Salmonella in meat and poultry products*, Food Research International, 45(2), pp. 713–721.

McFeeters, R.F. (1993) *Single-injection HPLC analysis of acids, sugars, and alcohols in cucumber fermentations*, Journal of Agricultural and Food Chemistry, 41(9), pp. 1439–1443.

McGlynn, W. (2016). *The Importance of Food pH in Commercial Canning Operations* [Online]. Available at: http://extension.okstate.edu. (Accessed date: 25 August 2022).

Montville, T.J., Matthews, K.R. and Kniel, K.E. (2012). *Food microbiology: An introduction.* 3rd ed. Washington, DC: ASM Press.

Nabavi, S., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E. and Daglia, M. (2015) *Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical ındustries,* Nutrients, *7*(9), pp. 7729–7748.

Nadia, Z. and Rachid, M. (2013) *Antioxidant and antibacterial activities of Thymus vulgaris L.,* Medicinal and Aromatic Plant Research Journal, 1(1), pp. 5–11.

Novik, G., Meerovskaya, O. and Savich, V. (2017) *Waste Degradation and Utilization by Lactic Acid Bacteria: Use of Lactic Acid Bacteria in Production of Food Additives, Bioenergy and Biogas.* London: IntechOpen, Available at: https://www.intechopen.com/chapters/55776 E-Book. (Accessed: 25 August 2022).

Pang, X., Song, X., Chen, M., Tian, S., Lu, Z., Sun, J., Li, X., Lu, Y. and Yuk, H. (2022) *Combating biofilms of foodborne pathogens with bacteriocins by lactic acid bacteria in the food industry,* Comprehensive Reviews in Food Science and Food Safety, 21(2), pp. 1657–1676.

Perez-Diaz, I.M., Breidt, F., Buescher, R.W., Arroyo-Lopez, F.N., Jimenez-Diaz, R., Fernandez, A.G., Gallego, J.B. and Yoon, S.S. (2009) *Compendium of Methods for the Microbiological Examination of Foods*. Fermented and acidified vegetables. New York: APHA Press, Available at: https://ajph.aphapublications.org/doi/epdf/10.2105/MBEF.0222.056. (Accessed: 25 August 2022).

Prasirtsak, B., Thitiprasert, S., Tolieng, V., Assabumrungrat, S., Tanasupawat, S. and Thongchul, N. (2019) *D-Lactic acid fermentation performance and the enzyme activity of a novel bacterium Terrilactibacillus laevilacticus SK5–6,* Annals of Microbiology, 69(13), pp. 1537–1546.

Putnik, P., Pavlic, B., Sojic, B., Zavadlav, S., Zuntar, I., Kao, L., Kitonic, D. and Kovacevic, D.B. (2020) *Innovative Hurdle Technologies for the Preservation of Functional Fruit Juices*, Foods, 9(6), pp. 699.

Ray, R.C. and Joshi, V.K. (2014) *Fermented Foods: Past, Present and Future* in Microorganisms and Fermentation of Traditional Foods (ed.), Florida: CRC Press, pp. 14-36. Available at: https://www.taylorfrancis.com/chapters/edit/10.1201/b17307-4/fermented-foods-past-present-future-ramesh-ray-joshi. (Accessed: 25 August 2022).

Sabra, W., Quitmann, H., Zeng, A.P., Dai, J.Y., Xiu, Z.L. (2011) *Microbial production of 2,3-Butanediol,* Elsevier Comprehensive Biotechnology, pp. 87–97.

Isbilir, S. and Sagiroglu, A. (2011) *Antioxidant potential of different dill (Anethum graveolens l.) leaf extracts,* International Journal of Food Properties, 14(4), pp. 894–902.

Sezey, M. and Adun, P. (2019) *Validation of mohr titration method to determine salt in olive and olive brine*, Journal of the Turkish Chemical Society Section A: Chemistry, 6(3), pp. 329–334.

Shyu, Y.S., Lin, J.T., Chang, Y.T., Chiang, C.J. and Yang, D.J. (2009) *Evaluation of antioxidant ability of ethanolic extract from dill (Anethum graveolens L.) flower*, Food Chemistry, 115(2), pp. 515–521.

Simbine, E.O., Rodrigues, L.C., Burbarelli, M.F.C., Favaro-Trindade, C.S., Viegas, E.M.M., Enke, D.B.S. and Lapa-Guimaraes, J. (2022) *Cinnamomum zeylanicum extracts reduce lipid oxidation in broadband anchovy (Anchoviella lepidentostole) minced fish*, Food Science and Technology, 41(3), pp. 643-652.

Singh, B., Chandra, S., Kumar, T., Kumar, R., Kumar, V., Chaudhary, V., Kumar, R. and Correspondence, B. (2019) *Performance evaluation and energy consumption analysis of dairy industry by milk pasteurization*, Journal of Pharmacognosy and Phytochemistry, 8(2), pp. 2236-2240.

Soyer, F., Keman, D., Eroğlu, E. and Türe, H. (2020) *Synergistic antimicrobial effects of activated lactoferrin and rosemary extract in vitro and potential application in meat storage,* Journal of Food Science and Technology, 57(12), pp. 4395–4403.

Suppakul, P., Miltz, J., Sonneveld, K. and Bigger, S.W. (2003) *Antimicrobial properties of basil and its possible application in food packaging*, Journal of Agricultural and Food Chemistry, 51(11), pp. 3197–3207.

Swain, M.R., Anandharaj, M., Ray, R.C. and Parveen Rani, R. (2014) *Fermented fruits and vegetables of Asia: a potential source of probiotics,* Biotechnology Research International [Online]. Available at: https://downloads.hindawi.com/archive/2014/250424.pdf (Accessed: 26 August 2022).

Takwa, S., Caleja, C., Barreira, J.C.M., Soković, M., Achour, L., Barros, L. and Ferreira, I.C.F.R. (2018) *Arbutus unedo L. and Ocimum basilicum L. as sources of natural preservatives for food industry: A case study using loaf bread*, LWT, 88, pp. 47–55.

Testa, B., Lombardi, S., Macciola, E., Succi, M., Tremonte, P. and Iorizzo, M. (2019) *Efficacy of olive leaf extract (Olea europaea L. cv Gentile di Larino) in marinated Anchovies (Engraulis encrasicolus, L.) process,* Heliyon, 5(5).

Safefood 360. (2014). *Thermal Processing of Food*. [Online]. Available: http://www.tiselab.com/pdf/Thermal-Processing-of-Food.pdf. (Accessed: 25 August 2022).

Tur, J.A. and Bibiloni, M.M. (2016), *Functional foods,* Encyclopedia of Food and Health, pp. 157–161.

Turgis, M., Han, J., Caillet, S. and Lacroix, M. (2009) *Antimicrobial activity of mustard essential oil against Escherichia coli O157:H7 and Salmonella typhi,* Food Control, 20(12), pp. 1073–1079.

Uylaşer, V. and Başoğlu, F. (2016) *Temel Gıda Analizleri*. Bursa: Dora Basım Yayın.

Veenstra, J.P. and Johnson, J.J. (2021) *Rosemary (Salvia rosmarinus): Health-promoting benefits and food preservative properties*, International Journal of Nutrition, 6(4), pp. 1–10.

Vidanagamage, S.A., Pathiraje, P.M.H.D. and Perera, O.D.A.N. (2016) *Effects of Cinnamon (Cinnamomum Verum) Extract on functional properties of butter,* Procedia Food Science, 6, pp. 136–142.

Viji, P., Binsi, P.K., Visnuvinayagam, S., Bindu, J., Ravishankar, C.N. and Srinivasa Gopal, T.K. (2015) *Efficacy of mint (Mentha arvensis) leaf and citrus (Citrus aurantium) peel extracts as natural preservatives for shelf life extension of chill stored Indian mackerel,* Journal of Food Science and Technology, 52(10), pp. 6278–6289.

Voldrich, M., Horsakova, I., Cerovsky, M., Cizkova, H. and Opatova, H. (2009) *Factors affecting the softening of pickled pasteurised cucumbers,* Czech Journal of Food Sciences, 27(1), pp. 314–318.

Yaralı, E. (2017). '*Gıdalarda Temel İşlemler*' Adnan Menderes University Unit Operations in Food II lecture notes, Aydın.

Yavuz, N., Foster, L., Sharma, T., Patel, K., Stoforos, G., Sandeep, K., Palnitkar, P. and Breidt, F. (2018) *Hot-fill pasteurization of cucumber pickle spears: An alternative to tunnel pasteurization,* Food Protection Trends, 38(4), pp. 258–265.

Yi, L., Qi, T., Hong, Y., Deng, L. and Zeng, K. (2020) *Screening of bacteriocin-producing lactic acid bacteria in Chinese homemade pickle and dry-cured meat, and* *bacteriocin identification by genome sequencing,* LWT - Food Science and Technology, 125, pp. 109177.

Yilmaz, E.S., Timur, M. and Aslim, B. (2013) *Antimicrobial, antioxidant activity of the essential oil of bay laurel from Hatay, Turkey,* Journal of Essential Oil Bearing Plants, 16(1), pp. 108–116.

Zenker, M., Heinz, V. and Knorr, D. (2003) *Application of ultrasound-assisted thermal processing for preservation and quality retention of liquid foods,* Journal of Food Protection, 66(9), pp. 1642–1649.