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作品名稱 **New Approach to Food Packaging: Antimicrobial and Edible Materials**

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關鍵詞 **Nisin、edible packaging、antimicrobial packaging**

作者照片

1.Purpose

In addition to adequate and balanced nutrition in the protection of human health, the reliability of consumed foods is also of great importance. When foods contact with the environment, they are undergoing many microbiological, physical and chemical changes such as moisture loss, aroma exchange, oxidation and contamination with microorganisms. So this changes reduce quality and shorten the shelf life. During cutting and processing of chicken meat, especially contamination on the surface causes deterioration of the meat starting from the surface and thus short shelf life. Though contamination of chicken meat surface is inevitable, growth of contaminant microorganisms can be inhibited and microorganisms can be killed. In recent years, increased risks of infection due to antibiotic-resistant microorganisms have forced the discovery of new and natural antibacterial materials. It is a new and advantageous approach to avoid environmental pollution caused by the use of food packaging and safety of food, prolongation of product shelf life by natural, edible, antibacterial biomolecules in packaging products.

In the scope of the project, in order to produce an edible alginate gel containing the antimicrobial peptide nisin, which has an antibacterial property to prevent microbial growth on the surface of chicken meat, following steps was done;

- ✓ Synthesis of calcium alginate and nisin immobilized calcium alginate beads
- ✓ Optimization of immobilizing nisin in calcium alginate beads,
- ✓ Characterization of nisin immobilized calcium alginate beads by ATR-FTIR Spectrum and SEM Analysis,
- ✓ Determination of the antimicrobial activity of white meat product chicken which is coated by nisin immobilizing calcium alginate gel.

2. Introduction

2.1. Antimicrobial Packaging

Foods are packaged to prevent biological, physical and chemical contamination as well as to protect against external factors such as oxygen, light and water. The pore size of the packaging materials, moisture and gases in the outdoor environment determine the quality and shelf life of the foods. Traditional methods (drying, freezing, heat treatment, etc.) and packaging methods serve as barriers to protect food from external factors. In addition to the barrier function of these packages, active packaging systems have been developed with the addition of various components to the packaging, the use of functional polymers in order to control the environment in the packaging and to participate in some other responsive functions (Purma et al., 2006; Gök, 2011). Active packaging methods are oxygen scavengers, carbon dioxide and moisture regulators, with use of antioxidants, antimicrobial packaging (Bagdatli, 2010). The antimicrobial packaging, which emerges as a new active packaging technique due to the consumers having minimum processed, high quality and safe food requirements, aims to provide food safety and extend the shelf life of meat and meat products in particular. Antimicrobial agents are added to the packaging material or the package cavity to reduction, inhibition of microorganisms in food and packaging materials (Mastromatteo, 2014). In antimicrobial packaging, packaging material and antimicrobial agents are used together to control the microbial growth on the food surface. Antimicrobial compounds in the packaging material are included in the formulation of the film, the packaging material is

coated with the antimicrobial agent to be used, or the antimicrobial agent is immobilized in the packaging material. The addition of natural antimicrobials to edible biopolymer films and coatings is an important practice (Karagöz and Candogan, 2007).

2.2. Antimicrobial Packaging Methods

When antimicrobial packaging techniques are applied, care is taken to ensure that the antimicrobial agent is homogeneously dispersed in the film, does not change the film barrier and mechanical properties, and that the film antimicrobial activity is high.In antimicrobial packaging which polymeric structures are used, antimicrobial release depends on the type, composition and method of application of the polymer (Mastromatteo et al., 2014, Appendini et al., 2002, Balasubramanian et al., 2009).

The techniques used in antimicrobial packaging are as follows:

- ✓ **Sachets / pads containing antimicrobial agents,** oxygen absorbers, moisture absorbers and ethanol vapor producers are the most commonly used forms in this regard. In order to prevent oxidation and moisture condensation in oxygen and moisture retainers, ethanol-producing systems are used to create protective effects on food. Oxygen scavengers prevent the growth of aerobic bacteria and molds by reducing the oxygen content in the pack.Moisture trapping inhibits microbial growth by decreasing water activity by retaining the nematode as a result of temperature fluctuations, especially in frozen products and temperature fluctuations in food stored in highly relative humid environments (Appendini et al., 2002, Suppakul et al., 2003).
- ✓ **The addition of antimicrobials to polymers** has attracted considerable interest in recent years in the food industry for the packaging films which is obtained by the addition of different antimicrobials (lysozyme, organic acids, essential oils, etc.) to polymers. Addition of natural antimicrobials to the thermoplastics, thermoset, paper, and other materials inhibits growing of Listeria monocytogenes, pathogenic Escherichia coli (Appendini et al., 2002). In packaging materials where non-volatile antimicrobial materials are used, multilayer films are used for slow diffusion of antimicrobial material into food. The inner layer controls the diffusion rate of the active ingredients, the matrix layer contains the active ingredients and the barrier layer prevents the antimicrobial materials from diffusing out of the packaging material (Ayana and Turhan, 2010).
- ✓ **Coating or adsorbing the antimicrobial material onto the polymer surface,** the addition of sorbic acid to packaging materials such as paper, cellulosic sheath, and the like is used frequently, especially in waxes used in fruit and vegetable coatings. However, antimicrobials, sensitive to the temperature applied in the processing of the polymer materials used in food packaging are coated onto the surface after the production of the packaging material. In this context, antimicrobial carrier edible films used as coatings for food or packaging materials can also provide control of moisture, gas and solid mobility (Appendini et al., 2002). Surface adhesion of antimicrobials depends on properties such as concentration of coating, hydrophilic character and production procedures. In addition, food properties such as storage conditions such as temperature and time, pH, water activity affect the yield of antimicrobial packaging (Olivas et al., 2009).
- ✓ **Immobilization of antimicrobials with ionic or covalent bonds to polymers,** in this context, antimicrobials such as peptides, enzymes, polyamines and organic acids are

immobilized to polymers such as polystyrene, polyvinyl chloride, ethylene vinylacetate (Appendini ve ark., 2002).

- ✓ **The use of polymers with natural antimicrobial properties, such as chitosan,** poly-L-lysine, are natural antimicrobial properties. The cationic charges in the chitosan compete with calcium in the electronegative regions of the membrane surface, thereby blocking bacterial entry into the cell and causing bacterial death (Appendini et al., 2002).

2.3. Use of Antimicrobial Packaging in Foods

It is preferred in antimicrobial packaging, cheese, fruit-vegetable, meat, fish, poultry products, bakery products; antimicrobial substances such as organic acids and salts, sulfites, nitrites, antibiotics, alcohols, enzymes, gasses, essential oils, bacteriocins and ethylene diamine tetraacetic acid are used for this purpose. Coatings and films have been used extensively in packaging in recent years because antimicrobial agents penetrate into food over time and thus provide antimicrobial action for a longer period of time (Mastromatteo et al., 2014). When studies in the literature are examined, there are studies investigating the effects of essential oils used on microorganisms in edible films and coatings

In the study by Carolina et al., Antimicrobial effects of packages obtained from the addition of low-density polyethylene to partridge and thyme essential oils were detected in E. coli O157: H7, Salmonella typhimurium and L. monocytogenes (Carolina et al., 2012).

In addition, organic acids are also preferred for antimicrobial packaging with low cost. In the study conducted in 2011, it was determined that sorbic acid-coated antimicrobial films coated with E. coli-infected Gouda cheese and pig filetosa cause a decrease in the number of E. coli in the samples (Hauser et al., 2011).

Antimicrobial peptides (AMP), another antimicrobial agent, bacteriocins produced by bacteria, can be used in packaging materials. In a study conducted by Mauriello et al. In 2005, it was determined that commonly used nisin-coated plastic films cause a decrease in the number of Micrococcus luteus in raw and pasteurized milk.

In addition, it has been shown that the use of polyethylene edible films containing nisin and other bacteriocins inhibits the development of L. monocytogenes, particularly in meat products (Mauriello et al., 2004, Pérez-Pérez et al., 2006, Dawson et al., 2002). In a different study, natamycin-impregnated cellulose films were found to be effective in preventing mold growth on the cheese surface (Mastromatteo et al., 2014).

2.4. Edible Film and Coatings

Edible films and coatings; are environmentally friendly materials made from natural sources, not synthetic, thin layer formed on the surface of a food. The use of edible film/coating materials makes it possible to simplify and reduce the packaging material required for food products, as well as the risk of cancer risk and environmental pollution, which are serious problems with food packaging made with plastics. In line with these needs, the developments in edible films and coatings have gained momentum, especially in the 2000s.While hydrocolloids (protein and polysaccharide) and lipids are mainly used in the preparation of edible films, it is possible to utilize composite films obtained from their combinations (Küçük et al., 2017).

An edible filmin with good quality; it is important to be stable against the physical and biochemical reactions that can occur between the barrier properties (moisture, oxygen permeabilities), the film and/or the atmosphere and the film, as well as their sensory properties (transparent, tasteless and odorless), reliable, environmental friendly and cost-effective in terms of health. For these reasons, edible films and coatings generally utilize polysaccharide-based products such as starch, cellulose and derivatives, gums(guar, locust bean, carrageenan, pectins and other derivatives) and chitin/chitosan and alginate (Sarıkuş, 2006). In recent years, edible films and coatings, which are frequently used in research, are derived from polysaccharide-based alginate, alginic acid and alginates from brown seaweed (Norajit et al 2010; Rhim et al 1998). Film and coatings obtained from alginates prevent moisture loss in food when used as packaging material and reduce the aggravation of lipid oxidation (Draget et al 1995; Fabra et al 2008a; Fabra et al 2008b). In addition, naturally derived alginate is both environmentally friendly and economically valued. Alginate is used in many food products to increase the shelf life and to reduce quality losses and new methods are being developed continuously. No allergic effects of alginate-based films have been found, and the natural result is that alginate is both environmentally friendly and economically advantageous (Yeşiltaş 2012).

2.5. Antimicrobial Peptides (AMP)

AMPs are cationic molecules containing 10-25 amino acids and having a molecular weight of 1-5 kDa (Van't Hof et al., 2001). AMPs can exhibit bactericidal, fungicidal and virucidal effects at very low concentrations, and the probability of bacterial resistance to these peptide molecules is very low. Because they are present in the immune systems of multicellular microorganisms, they can be purified from natural sources (Reddy et al., 2004). The mechanism of action of AMPs is thought to be due to their physicochemical properties, which interact with bacterial membranes containing lipid-dense and negatively charged lipid groups and cause the death of bacteria by breaking down the cell wall.

Studies in the literature have investigated the effect of covalent immobilization of AMPs on the surface and its effect on antimicrobial activity in order to prevent peptide aggregation and loss of proteolytic degradation after activation. In the study of Haynie et al., Magainin II AMP has been shown to exhibit bactericidal effects resulting from interaction with membranes of Gram-positive and Gram-negative bacteria by immobilizing to the polyamide resin surface (Haynie et al., 1995). In another study, biofilm formation was investigated by AMP immobilization on stainless steel surfaces, resulting in decreased bacterial adhesion on modified surfaces (Hequet et al., 2011). In the study of Willcox et al. 2008, antimicrobial activity of the peptide construct was investigated by adsorbing or covalently immobilizing mylimine, a synthetic peptide, on the surface of commercial contact lenses. It has been shown that peptide activity is higher in covalent immobilization over free amino groups present in the peptide structure, whereas coagulant activity of peptides adsorbing to the surface is lowered. It has been determined that these films, prepared in a study in which polyethylene films were modified with AMP, slowed down E. coli reproduction (Steven et al., 2008).

In addition, the effects of parameters such as immobilized AMP concentration, spacer molecule and chemical immobilization method on the biomolecule surface of antimicrobial activity in surface covalent immobilization of AMPs have been studied (Costa et al., 2011). PEG structures with a molecular weight of 3000 to 5400 kDa are used as intermediate molecules in the surface immobilization of AMPs (Costa et al. 2011). It has been suggested that by binding the AMP molecule to the intermediate molecule, it can interact with the bacterial membrane more easily and effectively.

PEG gives adhesion to surfaces, and nonspecific protein adsorption can be prevented by using PEG. In Gabriel et al.'s work in 2006, immobilization of LL37 AMPs on titanium surfaces was performed and immobilized surfaces in the presence of PEG were fatal for E. coli, but the same effect was not seen in the absence of PEG. When the surface immobilized peptide concentration on the activity of AMP was examined, it was determined by Glinel et al. that the bactericidal activity on Magainin I immobilized on 2- (2-methoxyethoxy) ethyl methacrylate was not decreased by the decrease in density. In a study conducted by Chen et al., increased antibacterial activity was observed in high concentrations of AMP (Glinel et al., 2009; Chen et al., 2009). When literature data are evaluated, it is expected that the immobilization of AMPs on different surfaces will enable the synthesis and widespread use of stable biomaterials with antimicrobial activity in which bacterial colonization and biofilm formation are inhibited.

2.5.1. Nisin Antimicrobial Peptide

The 3 different species of AMP, A, Z and Q contain 34 amino acids and 44% hydrophobic character. It acts on Gram positive bacteria (Antimicrobial peptide database, 2016). The best-known producer, Lactococcus lactis, is also produced by Streptococcus lactis. Nisin is produced in an inactive form containing 57 amino acids; 23 amino acids are separated from the N-terminus by dehydration, cyclization and peptide digestion, reaching a mature form of 34 amino acids.

Unlike many AMPs, it lacks the receptor to bind to the bacterial membrane. It kills bacteria in two steps:

Inoculation of the cell wall: It interacts hydrophobically or electrostatically with anionic cell wall components such as teichoic acid, teichuronic acid and lipoteichoic acid, and crosses the cell wall.

Interaction with Lipid II: The nisin interacts with lipid II after the cell wall is up, followed by one of the following two killing mechanisms:

1. It may bind to lipid II and inhibit the formation of peptidoglycan network.
2. Nisin bound to the carbohydrate-pyrophosphate residue in lipid II from the N-terminus.

Thus the C-terminus of the nisin can enter the membrane and form pores with a diameter of 2 nm. Membrane permeability increases, membrane potential disruption; components such as amino acids, ions start to move out of the cell and cell death occurs (Punyauppa-path et al., 2015).

Nisin, which is a high peptide relative to other peptide production yields; It is used in many sectors, from health to food, from agriculture to textiles.

In this study, it was preferred to use microbial chicken meat, which was rapidly degraded due to post-cutting processes. During cutting and processing of chicken meat, contamination

especially on the surface parts causes deterioration starting from meat surface. Microbial activities in chicken meat cause short shelf life of chicken meat and products. Though contamination of chicken meat surface is inevitable, growth of contaminant microorganisms can be prevented or these microorganisms can be killed. For this purpose, chicken meat is packaged in edible alginate gel containing nisin and microbial growth on the surface of chicken meat is avoided. There is no objection to the consumption of nisin and alginate by humans. Nisin is added as a natural preservative to milk and dairy products as food additive with E-234 code. Alginate is also used as a drug in the neutralization of stomach acid, especially in people with ulcers, as well as as food additive with E-400 code in food products.

3. Method

3.1. Materials

3.1.1 Chemicals

Potassium dihydrogen phosphate, nisin, sodium alginate, calcium chloride from Sigma; nutrient agar, peptone Fluka.

3.1.2 Devices

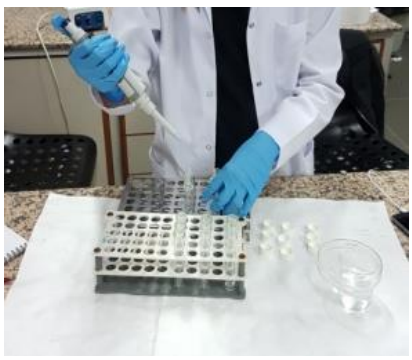
Devices used in the project; analytical balance (DAIHAN Biomedical), heating magnetic stirrer (IKA), pH meter (HANNA HI 2211-02), incubator (NEW BRUNSWICK SCIENTIFIC; STUART SCIENTIFIC), micropipette (EPPENDORF RESEARCH PLUS), water bath (MEMMERT), vortex (FISONS, WhirliMixer), microplate reader (THERMO SCIENTIFIC, Multiskan GO), sterile cabinet (ESCO CLASS 2 BSC), -86°C freezer (THERMO) and autoclave (HIRAYAMA).

Experiments within the scope of the project were carried out in the laboratories of Ege University Biochemistry Department of Science Faculty.

3.2. Immobilization of Nisin in Calcium Alginate

Sodium alginate was prepared by dissolving in 4 ml of distilled water at room temperature as 1.5%. 20 mg nisin (1 mL) prepared by dissolving in 25 mM pH 4 phosphate buffer was added. The solution was allowed to stand at +4°C for 30 minutes to remove air bubbles in the solution. This solution was added dropwise using a 3% $CaCl_2$ solubilising blunt needle prepared by injection of 2 ml and held at +4°C. The process was carried out in ice cubes at a stirring speed of 50 rpm. The nisin immobilized calcium alginate beads formed after dripping were left in the ice bath for 30 minutes. Finally, the nisin immobilized calcium alginate beads were washed with 1 ml of d. water for 10 minutes at a stirring rate of 150 rpm at 37 ° C. Washing was carried out in 3 replicates.

Figure 1: Immobilization of Nisin in Calcium Alginate



3.3. Quantitative Determination of Nisin

Immobilization was followed by the spectrophotometric determination of the $CaCl_2$ solution in which the wash water and bead forming process was carried out.The nisin was determined by absorbance at 215 nm. The washing water of the non-immobilized calcium alginate bead was used as a blank solution for the identification of the nisin. For the identification of the nisin from the $CaCl_2$ solution in which the bead forming process was carried out, the $CaCl_2$ solution was used as blank solution, in which beads were formed by dropwise addition of nisine-free sodium alginate solution.

Figure 2: Quantitative Determination of Nisin

3.4. Optimization of Immobilization of Nisin to Calcium Alginate Beads

Parameters for the optimization of immobilization of nisin to calcium alginate beads: Percentage of alginate (1.0-1.5-2.0-2.5-3.0), Percentage of $CaCl_2$ (0,5-1,0-3,0-5,0-7,5), amount of nisin (5-10-15-20-25 mg), number of beads (3-5-7-10-15-20), stirring time (0-15-30-60-120 min), temperature (4-15-25-37-55 °C) and the stirring speed (0-100-150-200-250 rpm).

3.5. Characterization of Nisin Immobilized Alginate Beads by ATR-FTIR Spectrum and SEM Analysis

Scanning electron microscopy (FEI QUANTA 250 FEG SEM) was used to image the surfaces of calcium alginate and nisin immobilized calcium alginate beads. Samples were imaged at 8-20 kV in argon atmospheres after gold plating.

The PERKIN ELMER SPECTRUM TWO series instrument was used for the FT-IR (Fourier transform infrared spectroscopy) spectrum. The spectra of calcium alginate and nisin immobilized calcium alginate beads were recorded at 4000-450 cm^{-1} using ATR (reduced total reflection) equipment.

3.6. Total Live Count

The potential application area of the product developed within the scope of the project is the food sector. For this reason, chicken meat was coated with nisin immobilized sample in optimum conditions and a total live count analysis of these products was performed. The reason for the selection of chicken meat as a product group; wide coverage in the sector, and chicken meat is a product open to microbial deterioration in terms of moisture and nutrient content. Samples treated with sodium alginate solution containing nisin and nisin-free sodium alginate solutions were subjected to total viable count analysis. Chicken specimens were first immersed in sodium alginate solution (with and without nisin) and applied with 3% $CaCl_2$ solution by spraying to give gel formation. Samples were incubated at +4°C for one week.

After one week, all samples were taken into a sterile stomacher bag and 0.1% sterile peptone solution was added and homogenized 3 times on a stomacher device at intervals of 30 seconds. Thus, 10^{-1} dilution solutions of the samples were prepared. Dilutions of 10^{-7} were prepared with 0.1% sterile peptone water from 10^{-1} dilution of sample. Samples prepared in all dilutions were seeded to petri with pour plate technique. The number of colony counts in the range of 40-300 petri was counted to determine the difference in total viable counts between nisin containing and noncontaining samples. The test was repeated as 3 samples in 5 samples. Calculations were made by taking the average of the results.

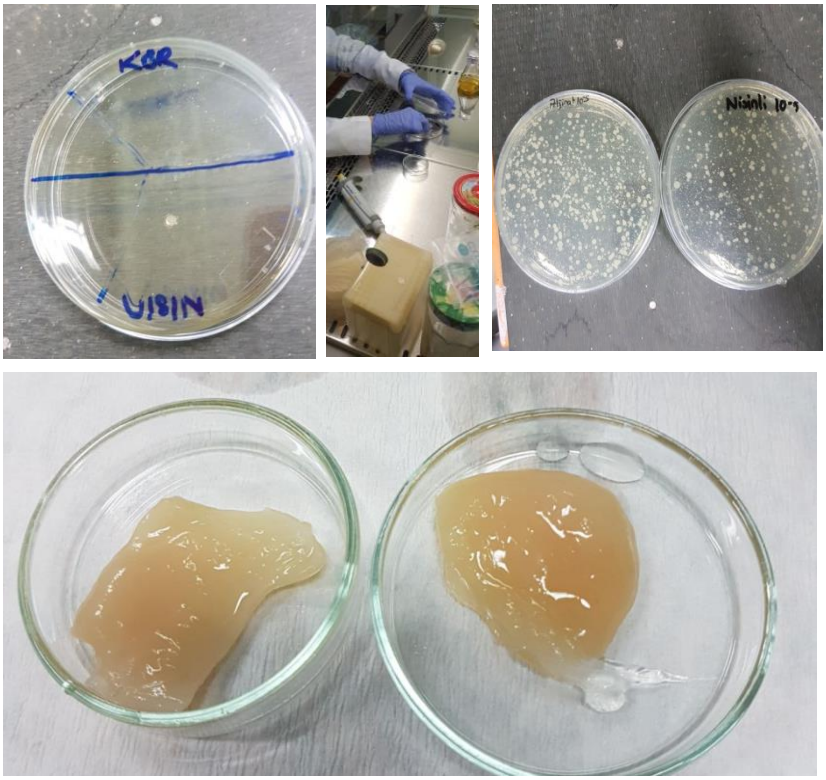
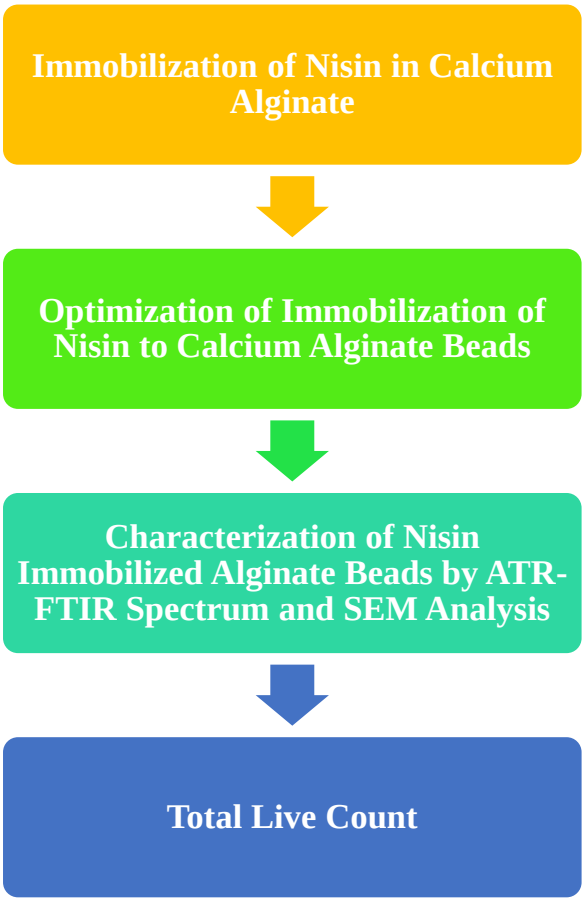


Figure 3: Total Live Count

3.7. Experimental Flow-chart



4. Results

4.1.Optimization of immobilization nisin to alginate

4.1.1. Alginate Percentage

The egg box model, formed between the alginate and Ca^{+2} ions, causes cross-linking gelation. Therefore, the two most important parameters affecting the nisin retention capacity of the formed gelatin are the percentage of alginate and Ca^{+2} ions (Won et al., 2005). The main parameter affecting the strength of calcium alginate gel is the alginate concentration and $CaCl_2$ concentration. Gelatin stability increases with increasing alginate concentration. However, the increase in alginate concentration also increases the viscosity, which prevents the formation of a homogeneous mixture (Göksungur and Güvenç, 2002). At the same time, as the alginate concentration increases, structural changes in the immobilized nisin area must be considered (Won et al., 2005). Therefore, the optimum percentage of alginate was tested and the optimum value was determined to be 1.5% (Fig. 4).

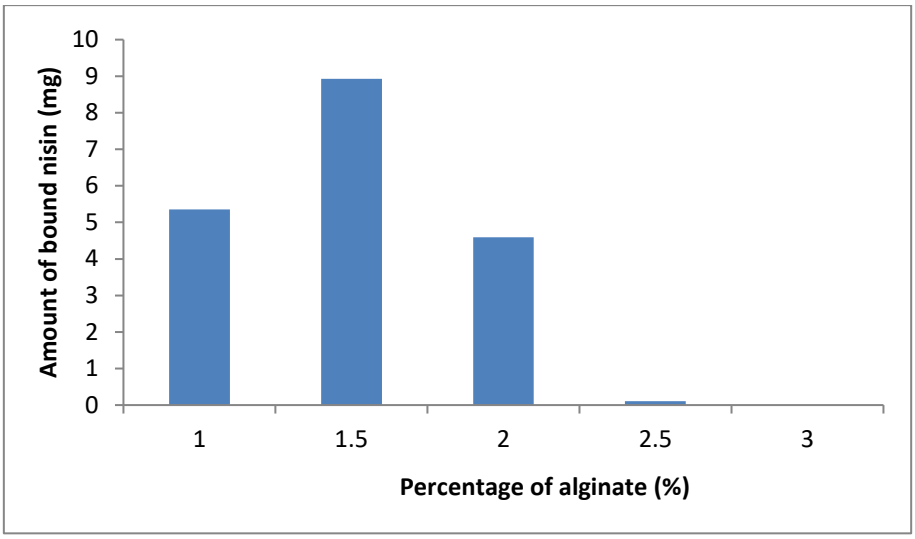


Figure 4: Effect of percentage of alginate for immobilization of nisin (Working conditions: 3% $CaCl_2$ percentage, 25 mg initial nisin amount, 5 beads, nisin immobilized calcium alginate beads in CaCl2 solution for 30 minutes, incubation temperature 25°C, stirring speed 150 rpm)

4.1.2. Calcium Chloride Percentage

There is a cross-link between α-L-guluronic acid and Ca^{+2} ions in the sodium alginate structure. If the ratio of Ca^{+2} ions in the medium is very low compared to α-L-guluronic acid, the gel structure is expected to be softer and brittle (Dong et al., 2017). Egg box model forms by displacement of Na^{+} ions in the structure of sodium alginate and Ca^{+2} ions in the $CaCl_2$ structure. In the case of excess calcium ions, these ions are replaced by a cationic AMP nisin, which leads to a reduction in the amount of immobilized nisin. For this reason, the optimum percentage of $CaCl_2$ should be determined. The optimum $CaCl_2$ percentage value was found to be 3% (Figure 5).

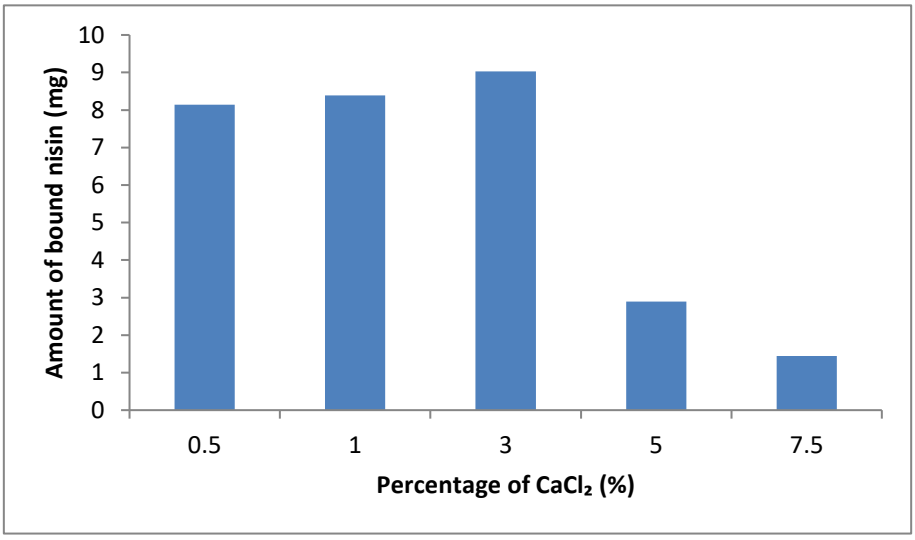


Figure 5: Effect of percentage of $CaCl_2$ on the immobilized of the nisin (Working conditions: 1.5% alginate percentage, 25 mg of inital nisin amount, 5 beads, immobilized calcium alginate beads in $CaCl_2$ solution for 30 minutes, incubation temperature 25°C, stirring speed 150 rpm)

4.1.3. Amount of Nisin

As the amount of nisin is increased, the amount of nisin which immobilized in calcium alginate beads will increase, but the because of cost is important factor especially in industrial projects, highest yield value should be chosen (Ozseker and Akkaya, 2016). The optimum amount of nisin was found to be 20 mg (Figure 6). Percentage of binding nisin is used when graph is being created. This value was obtained by multiplying the ratio of the amount of bound nisin to the amount of nisin added at the beginning of the reaction by 100. When 20 mg nisin was added to the medium, 40.16% (8.03 mg) of nisin in the reaction-ending medium was immobilized.

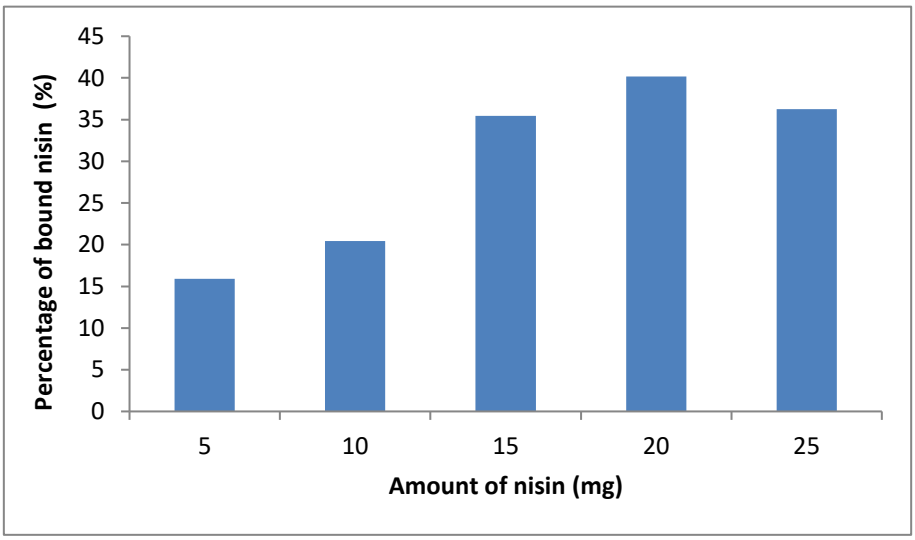


Figure 6: The effect of the amount of nisin to immobilization of nisin (Working conditions: 1.5% alginate percentage, 3% $CaCl_2$ percentage, 5 beads, nisin immobilized calcium alginate beads in $CaCl_2$ solution for 30 minutes, incubation temperature of 25°C, stirring speed of 150 rpm)

4.1.4. Number of beads

The optimum value for the test result, which was established to determine the effect of the number of beads for the immobilization of the nisin, was found as 10 beads. As can be seen from Fig. 7, the number of bound nisin increased as the number of beads increased. Although the increase in the number of beads does not affect the amount of nisin immobilization by the beads, such an increase is observed as more beads are taken from the medium and more nisin are taken.

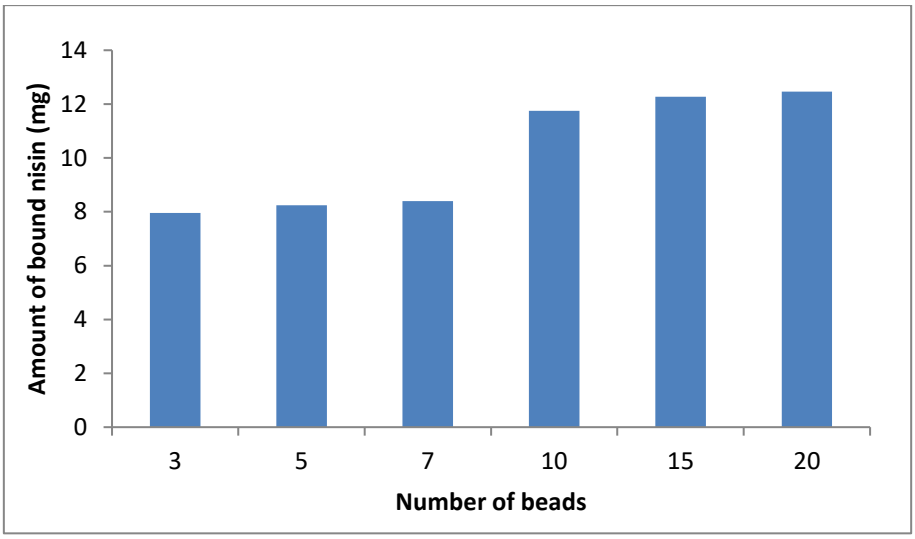


Figure 7: Effect of number of beads to immobilization of nisin (Working conditions: 1.5% alginate percentage, 3% $CaCl_2$ percentage, 20 mg initial amount of nisin, incubation time of immobilized calcium alginate beads in $CaCl_2$ solution for 30 minutes, incubation temperature 25°C, stirring speed 150 rpm)

4.1.5. Waiting Time in Nisin Immobilized Calcium Alginate Beads in $CaCl_2$ Solution

The substitution of Ca^{+2} ions in $CaCl_2$ and Na^{+} in the sodium alginate structure determines the strength and stiffness of the beads. The duration time of sodium alginate in $CaCl_2$ increases the strength and stiffness of the bead. It is also important to release nisin through the bead as long as the strength and hardness of the nisin immobilized calcium alginate bead is significant. As the duration time of stay in $CaCl_2$ increases, the release of nisin from the bead increases, so the amount of bound nisin decreases. For this reason, it is necessary to determine the optimum duration time of the beads in $CaCl_2$ (Muslu, 2016). As a result of the experiment, the optimum waiting time was found to be 30 minutes (Figure 8).

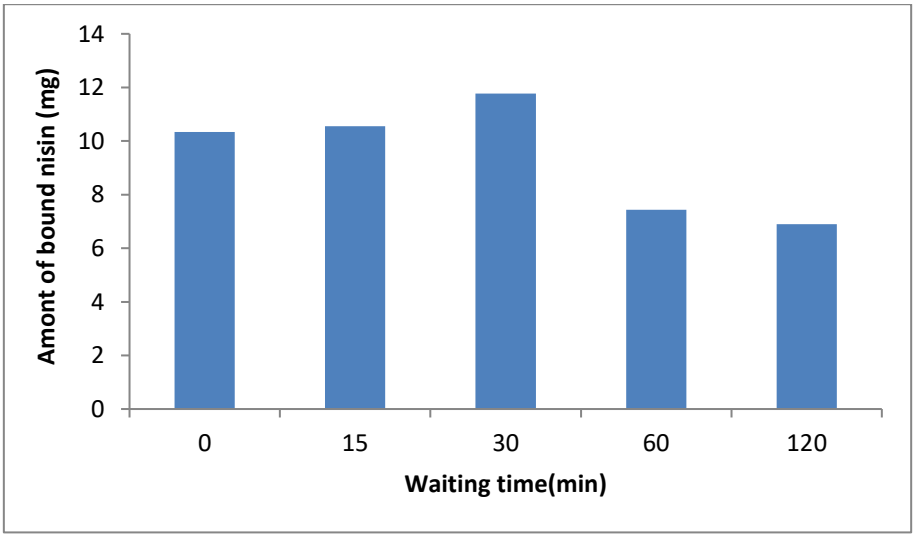


Figure 8: Effect of waiting time in CaCl2 solution to nisin immobilization (Working conditions: 1.5% alginate percentage, 3% $CaCl_2$ percentage, amount of initial nisin 20 mg, 10 beads, incubation temperature 25°C, stirring speed 150 rpm)

4.1.6. Temperature

As in most polysaccharide solutions, temperature increases in alginate solutions also reduce viscosity (Tezcan, 2008). Decrease in viscosity can lead to damage to the bead structure and allow the nisin to exit the bead quickly. In order to predict this damage, a temperature experiment was carried out and the optimum value was found to be 37°C (Figure 9).

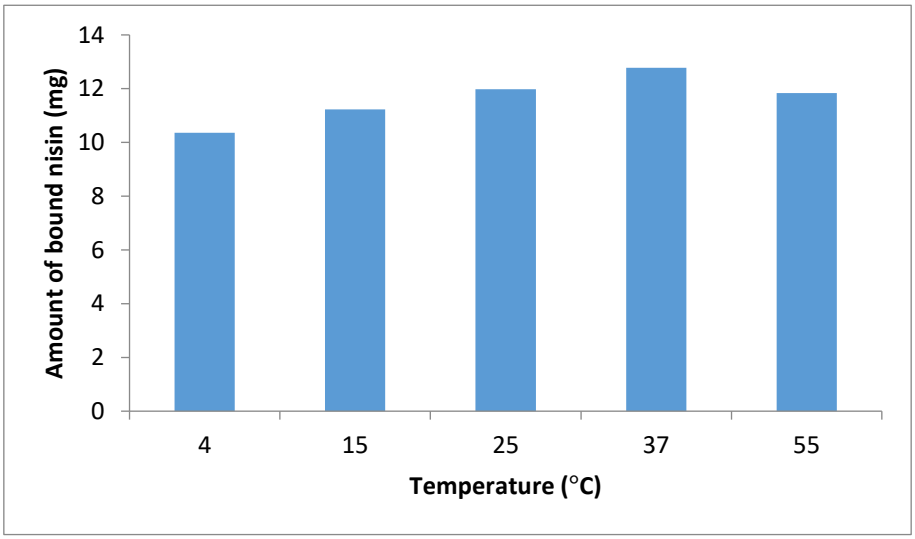


Figure 9: Effect of temperature to immobilization of nisin (Working conditions: 1.5% alginate percentage, 3% $CaCl_2$ percentage, 20 mg initial amount of nisin, 10 beads, incubation time of immobilized calcium alginate beads in $CaCl_2$ solution for 30 minutes, stirring speed 150 rpm)

4.1.7. Stirring speed

In order to determine the effect of stirring speed on immobilization, it has been determined in the experiment that stirring speed does not affect immobilization because of its relative nisin release (Turkmen, 2008) (Figure 10).

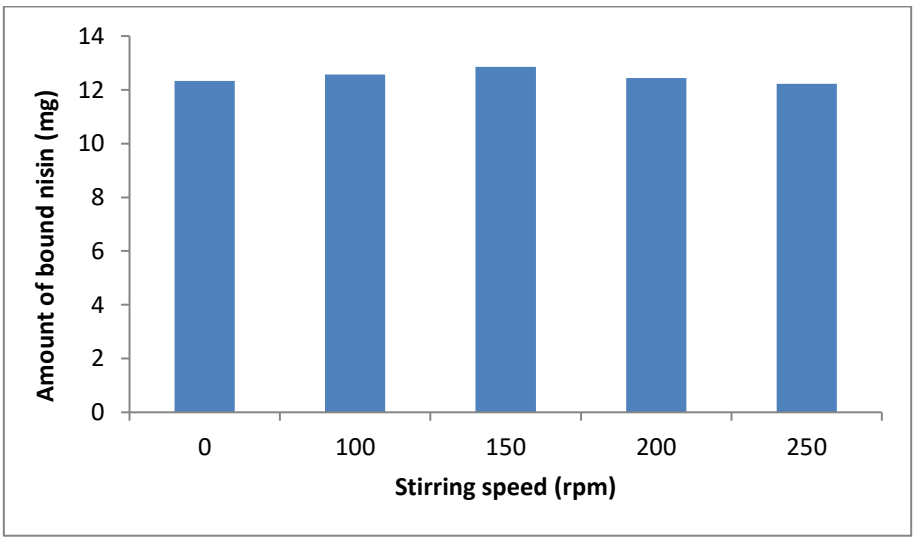


Figure10: Effect of stirring speed to immobilization of nisin (Working conditions: 1.5% alginate percentage, 3% $CaCl_2$ percentage, 20 mg initial amount of nisin, 10 beads, incubation time of immobilized calcium alginate beads in $CaCl_2$ solution for 30 minutes, incubation temperature 37°C)

4.2. ATR-FTIR Spectrum and SEM Images of Nisin Immobilized Calcium Alginate Beads

Characterization studies were carried out to demonstrate immobilization after completion of the optimization of immobilization in the project. At the characterization stage, the ATR-FTIR spectra and SEM images of nisin immobilized calcium alginate beads in optimum conditions and untreated beads were taken.

As a result of the ATR-FTIR spectrum, it was observed that there was no difference between the spectra of the samples which were not treated and immobilized natively under optimum conditions. The ATR-FTIR spectrum is formed from the surface of the beads. Immobilization has been carried out by trapping nisin in calcium alginate beads. For this reason, over time, the release of nisin from calcium alginate beads occurs. During the drying of the calcium alginate beads, the released nisin remained on the surface of the calcium alginate beads. However, no difference in the spectral results was obtained because of the amount of nisin residues on the surface of calcium alginate beads is very low (Fig. 11 and Fig. 12).

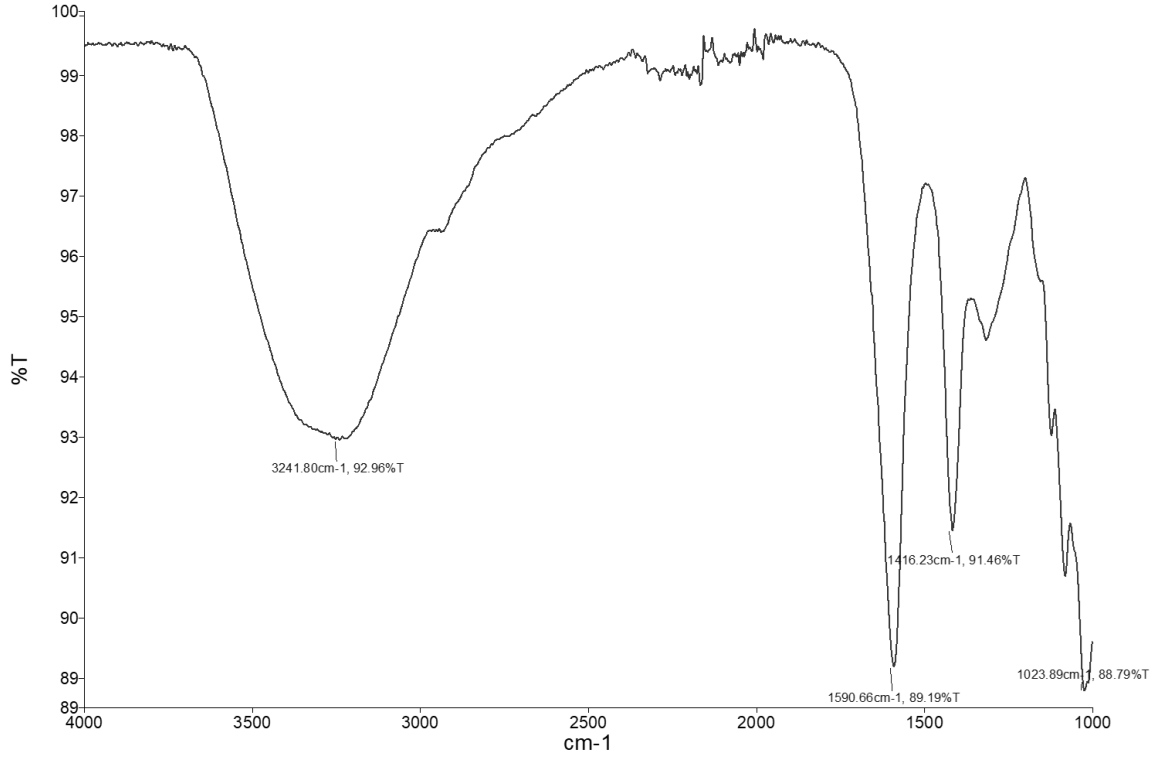


Figure11: ATR-FTIR spectrum of calcium alginate

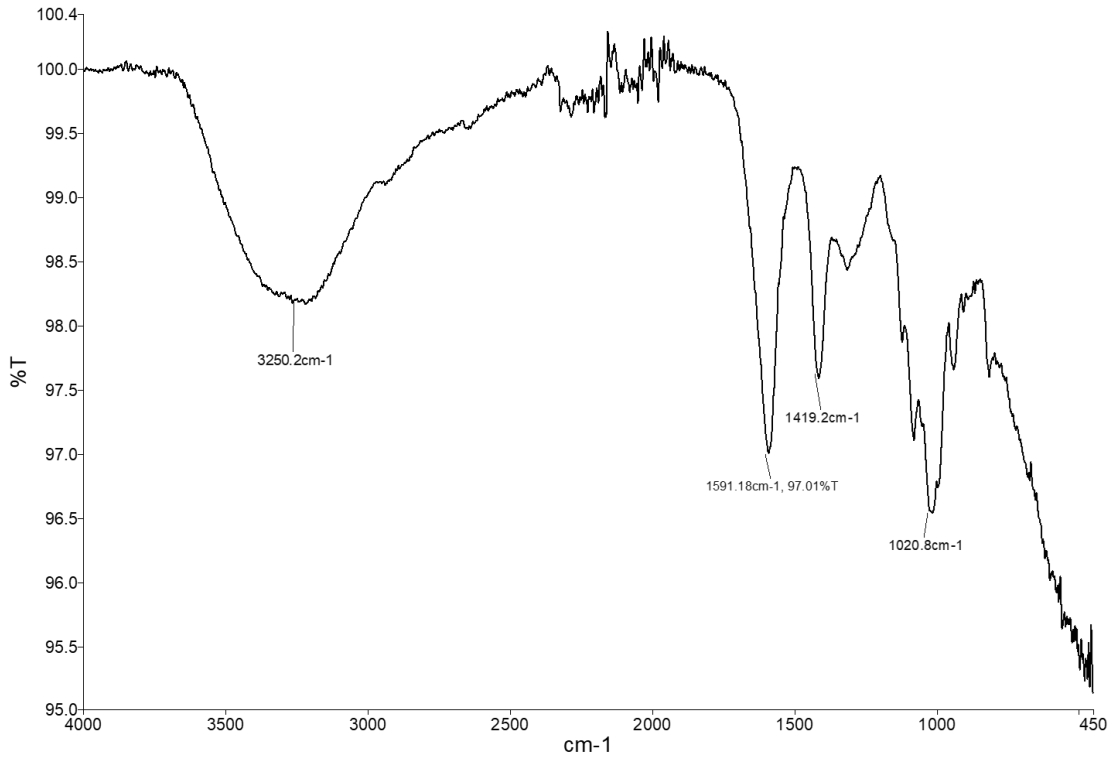


Figure 12: ATR-FTIR spectrum of nisin immobilized calcium alginate

In the study immobilization was carried out by trapping nisin on calcium alginate beads. For this reason, SEM images of calcium alginate bead are not expected to have additional structures originating from nisin. However, when the SEM image of the nisin immobilized calcium alginate bead is examined, there are some additional structures unlike the SEM image of the untreated calcium alginate bead (Figs. 13 and 14). These structures are thought to be originated from the immobilized nisin. Over time, the release of the trapped nisin in the calcium alginate beads occurs. The different structures seen in the SEM image of the nisin immobilized calcium alginate are believed to be due to the dryness of the nisin which released calcium alginate bead on surface during the drying of the nisin immobilized calcium alginate bead.

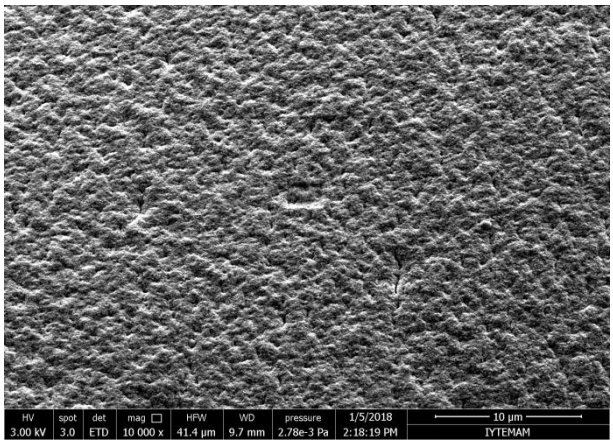


Figure 13: SEM image of untreated calcium alginate bead

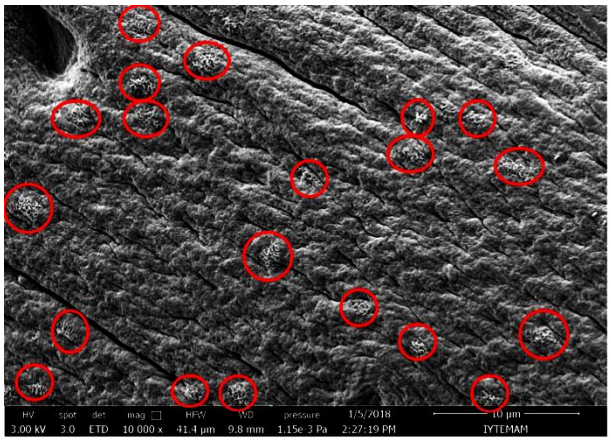


Figure 14: SEM image of nisin immobilized calcium alginate bead

4.3. Total Live Count

Antimicrobial packaging materials; are defined as materials that interact with the upper layer of the product or product to reduce, inhibit or delay the growth of microorganisms on the surface of food products. These materials are produced to prevent food contamination, to reduce the risk of pathogen development and to extend shelf life. The project aims to develop a packaging material with antimicrobial properties by immobilizing nisin to calcium alginate beads. The potential application area of the developed product is the food sector. For this reason, the sample which nisin immobilized in optimum conditions is applied to the chicken meat and the total count of live products applied to the food products on the market has been analyzed. The reason for the selection of chicken meat as a product group; wide coverage in the sector, a product open to microbial deterioration in terms of moisture and nutrient content. As a result of the analysis, it was observed that the developed product reduced the total number of living things by 90%.

5. Discussion

In addition to adequate and balanced nutrition in the protection of human health, the reliability of consumed foods is also important. Foods are undergoing many microbiological, physical and chemical changes such as moisture loss, aroma exchange, oxidation and contamination with microorganisms, which reduce quality at the same time shorten the shelf life when they are in contact with the environment. Various methods such as heating or cooling, lowering of water activity, curing, salting, pH control, addition of antimicrobial agent, controlled atmosphere storage and packaging are used to extend the shelf life of food and increase product quality. Food packaging applications help to increase the shelf life of food by providing the choice of packaging material and packaging technology suitable for different food products and enables to maintain the quality and freshness within this period. The materials used for the packaging of food mainly consist of paper, metal, glass and plastic. However, care should be taken to ensure that packaging materials are suitable for the composition of foods and that they do not cause environmental pollution.

In recent years, due to the fact that they have been made from edible materials, work on edible packing materials which are broken faster in the environment than other packaging materials has increased. Edible packaging materials generally consist of four basic materials, polysaccharide, lipid, protein and resins. Among them, polysaccharides edible coatings are preferred because of their carbon dioxide and oxygen permeability, thus providing the desired modified atmosphere condition without creating an anaerobic environment. Alginate, a polysaccharide derived from brown seaweed, is often used as edible packaging material because the prevents product from moisture loss and positively affects the aggravation of lipid oxidation. Alginate is one of the materials frequently used in the coating of aquatic products and meat products as edible films (Datta et al., 2008). In this study, it was concluded that chicken meat, which is a white meat product, was used together with nisin to create a synergistic effect.

Within the scope of the project, studies were carried out to optimize the immobilization of nisin sodium alginate. The amount of immobilized before the optimization studies was 8.93 mg, but after optimization studies this value was found to be 12.85 mg. As a result, the amount of immobilized nisin is increased by 43.9%. Immobilization has been proven by different methods (ATR-FTIR spectroscopy and SEM imaging) and the product has been subjected to total viable count analysis since it was designed as antibacterial.

The potential application area of the product developed within the scope of the project is the food sector. For this reason, the sample which nisin immobilized in optimum conditions is applied to the chicken meat and the total count of live products applied to the food products on the market has been analyzed. As a result of the analysis carried out, it was observed that in the case of nisin-containing sodium alginate, 90% of the total number of live was observed to be reduced.

Due to its wide coverage in the sector, it is a product that is open to microbial deterioration in terms of moisture and nutrient content, the sample application is made of chicken meat, but the developed product has the potential to be used for packaging different food products. The

antimicrobial properties of the product will increase shelf life. That is to say, the product will increase its potential to sell at the raft. This will result in a significant profit on the part of the manufacturer. Because the products that complete the shelf life are destroyed, both the company enters into a wound and this reflects negatively on the country's economy. It is predicted that the use of the developed product will significantly reduce the amount of product to be destroyed. In addition, in developed product, alginate selected as the coating material will not be a threat to the environment due to easy disintegration in the environment.

Because the nisin containing alginate gel completely covers the surface of the chicken meat, the contaminant microorganisms on the meat surface die because of nisin. Alginate is a hydrogel structured polymer and has very high water holding capacity. Since the chicken meat is covered with alginate gel, it does not have any water coming out of the meat, and as a result, it protects the appearance of the first cut of the meat after 1 week. The immobilization method used in the study is trapping method in hardenable gels. Nisin is sterically trapped in alginate gel and its motion is restricted. Nisin has released from the inside of the gel and has reached the surface of the chicken meat and killed contaminating microorganisms. It is obvious that the release will not be as fast as the nisin containing alginat the in solution. This is an advantage for the product. Because slow release slows down the proliferation of resistant microorganisms. Nisin is not only physically trapped in the gel but also has charge-based restriction of motion due to the electrostatic interaction between negative charged carboxylic acid groups in the alginate structure and positive charged amino acids in the nisin molecule. This means that the release is slower. Increasing the shelf life requires increasing the shelf life by at least 50% in order for a system or method to be successful. Since the microbial growth has been reduced by 90% with the product we have developed, it is thought that the target can be reached easily. Nisin is a peptide. Peptide bonds in the nisin structure, such as other proteins and peptides, are also hydrolyzed and amino acids are released. For this reason, there is no harm to the consumer. Since both nisin and alginate can be safely consumed by people, there will be no negative situation for human health when using the product developed as packaging material.

6. Implications

The antimicrobial property of the packaging materials developed within the scope of the project has been gained by using nisin. Different packaging materials can be developed by using antimicrobial agents other than nisin. It should be noted that the antimicrobial agent which can be added as a food additive. Because, in the developed product, antimicrobial agent is released and this agent is taken orally along with the consumer product. Nisin is an FDA-approved AMP used as a food additive in E-234 code on the market and therefore poses no consumer risk. Alginate was selected as a supporting material when the packaging material was developed in the project. According to the target product group, immobilization of different antimicrobial agents can be carried out on materials other than alginate. Thus different packaging materials can be obtained. As you can see, the project is a model for the development of alternative products to packaging materials on the market.The nisin used in the project is commercially provided. Nisin can be produced and purified from microorganisms and the obtained nisin after purification can be used for immobilization. The cost of the product developed in this way can be reduced.

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Nisin Antimicrobial Peptide is known to inhibit bacteria in two steps, inoculation of the cell wall and interaction with Lipid II. In this project, the authors aimed to use Nisin by immobilizing it on an edible alginate gel to prevent foods, i.e. chicken meat, from bacterial contamination. Strong points: They have tried different conditions to optimize the amount of nicin bound to chicken meat. They even used SEM to image nisin immobilized calcium alginate bead. These are thorough studies. The writing of this report was in the logical progression and was followed by the citation of proper scientific references.
Weakness: The results for the part of Total Live Count are very limited. They should test the shelf time for the chicken meat with Nisin. There is Fig. 3 in Method session, but no details shown as Results for this Fig. Even though they claim that there is no objection to the consumption of Nisin and alginate by humans (Nisin is added as a natural preservative to milk and dairy products as food additive with E-234 code), adding an antibiotic may still scare people when chicken meat was coated with Nisin immobilized. Is there toxicity test for Nisin? What is the tolerable dosage? If Nisin has been added as a natural preservative to milk and dairy products as food additive, what is the novelty of using Nisin in this study? Research comments: However, all experiments are recommended to perform at least three replicates from independent experiments, and proper statistical

comparisons may be needed in Figures 4-10. For scientific labels of digital numbers, one point five percentage shall be marked as 1.5% in your figures, but not 1,5%, for instance.