FABRICATION AND CHARACTERIZATION OF GELATIN BASED ACTIVE NANOFIBERS BY CENTRIFUGAL SPINNING

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ABSTRACT

FABRICATION AND CHARACTERIZATION OF GELATIN BASED ACTIVE NANOFIBERS BY CENTRIFUGAL SPINNING

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Centrifugal spinning is a novel method to produce fibers in a fast and efficient way to be used in active packaging. The objective of this study was to explore the impact of varying concentrations of caffeic acid and bay laurel leaf essential oil (BLLEO) on the characteristics of gelatin-based active fibers produced through centrifugal spinning. For BLLEO added samples, it was also aimed to optimize flow rate and fiber composition based on fiber diameter, antioxidant activity (AOA) and total phenolic content (TPC). In this aspect, different flow rates of solution (10, 15, 20 mL/h), concentrations of gelatin (20%, 25%) and concentrations of BLLEO (1%, 3%, 5%) were studied. Active gelatin fibers with different concentrations (control (0%), 2% and 3%) of caffeic acid and 1%, 3%, 5% of BLLEO were analyzed in terms of morphology, water vapor permeability (WVP), TPC, AOA, encapsulation efficiency (EE), Fourier Transform Infrared Spectroscopy (FTIR) and thermal analysis. Antimicrobial activity of fibers was also examined with two bacteria: *E. coli* and *S. aureus*. Morphological analysis showed that the addition of caffeic acid and BLLEO did not disrupt the homogenous structure of fibers. The AOA and TPC of fibers increased with the increase in active agent concentration. The application of both caffeic acid and BLLEO loaded fibers to olive oil during accelerated storage resulted in a notable reduction in total oxidation. In terms of TOTOX values, as compared to control sample, 3% caffeic acid added fibers 45% reduction, whereas 5% BLLEO added fibers had a reduction of 38%. Consequently, caffeic acid and BLLEO incorporated gelatin-based centrifugally

spun fibers might be a promising active packaging material due to their antioxidant or antimicrobial activities.

Keywords: Centrifugal Spinning, Gelatin, Caffeic Acid, Bay Laurel Leaf Essential Oil, Antioxidant Activity

ÖZ

MERKEZKAÇ KUVVETİYLE EĞİRME YÖNTEMİYLE JELATİN BAZLI AKTİF NANOFİBERLERİN ÜRETİLMESİ VE KARAKTERİZASYONU

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Santrifüj ile eğirme, aktif ambalajlamada kullanılan lifleri hızlı ve verimli bir şekilde üretmek için kullanılan yeni bir yöntemdir. Bu çalışmanın amacı, kafeik asit ve defne yaprağı esansiyel yağının (BLLEO) değişen konsantrasyonlarının santrifüjlü eğirme yoluyla üretilen jelatin bazlı aktif liflerin özellikleri üzerindeki etkilerinin araştırılmasıdır. BLLEO eklenen örnekler için, lif çapı, antioksidan aktivite (AOA) ve toplam fenolik içeriğe (TPC) dayalı akış hızının ve lif bileşiminin optimize edilmesi de amaçlanmıştır. Bu açıdan, farklı çözelti akış hızları (10, 15, 20 mL/saat), jelatin konsantrasyonları (%20, %25) ve BLLEO konsantrasyonları (%1, %3, %5) incelenmiştir. Farklı konsantrasyonlarda (kontrol (%0), %2 ve %3) kafeik asit ve BLLEO (%1, %3, %5) içeren aktif jelatin lifleri morfoloji, su buharı geçirgenliği (WVP), TPC, AOA, kapsülleme verimliliği (EE), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR) ve termal analiz açısından analiz edilmiştir. Liflerin antimikrobiyal aktivitesi *E. coli* ve *S. aureus* olmak üzere iki farklı bakteride incelenmiştir. Morfoloji analizi, kafeik asit ve BLLEO eklenmesinin liflerin homojen yapısını bozmadığını göstermiştir. Liflerin AOA ve TPC'si aktif madde konsantrasyonundaki artışla birlikte artmıştır. Hızlandırılmış depolama sırasında hem kafeik asit hem de BLLEO yüklü liflerin zeytinyağına uygulanması, toplam oksidasyonda önemli bir azalmayla sonuçlanmıştır. TOTOX değerleri kontrol örneği ile karşılaştırıldığında, %3 oranında kafeik asit eklenen liflerde %45 oranında azalma olurken, %5 oranında BLLEO eklenen liflerde sırasıyla %38 oranında azalma

görülmüştür. Sonuç olarak, kafeik asit ve BLLEO içeren jelatin bazlı santrifüjlü eğrilmiş lifler, antioksidan veya antimikrobiyal aktiviteleri nedeniyle umut vadeden bir aktif ambalaj malzemeleri olabilir.

Anahtar kelimeler: Santrifüjlü Eğirme, Jelatin, Kafeik Asit, Defne Yaprağı Esansiyel Yağı, Antioksidan Aktivite

To my family…

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CHAPTER 1

INTRODUCTION

1.1. Active Packaging

In food industry, packaging plays a crucial role in terms of providing food safety and hygiene in an economic way, by considering the environmental effects, customer, and industrial requirements. The functions of food packaging can be evaluated in four categories: Containment, protection, convenience, and communication. The first two of these are the main function against the deterioration caused by physical damage until the product arrives to the final consumer. Other than these, packages should show the content and the benefits of the product to get the attention of the consumer (Shlush and Davidovich-Pinhas 2022). Traditional food packages, made of plastic, are generally for single use and they have less than 20% recycle rate. Therefore, they possess a concern about environmental pollution due to their wastes. This concern resulted in a demand for biodegradable and sustainable packaging materials, which can also enhance food safety and quality. Alternative packaging polymers such as gums, starch, plant, and animal-based proteins can be used in active packaging systems (Trajkovska Petkoska et al. 2021).

Active packages are used to improve or maintain the food quality and safety by including active agents. European regulation (EC) No 450/2009 explains active packaging in two different types. The first one is the packaging material that can release active agents such as antioxidants, flavors, ethanol, and antimicrobial compounds to the food. On the other hand, the other one absorbs unwanted compounds such as water vapor, odor, ethylene, and oxygen from the surrounding of the food. Although these two different packaging systems act differently, the common aim is to reduce the deterioration of food quality and the spoilage of microorganisms (Guo et al. 2024). There are three parts of active packaging: active agents, packaging material and food. For the first part, as an active agent, antioxidants are commonly used in food industry. They can destroy the oxidation chain, so further oxidation reaction can be

obstructed. There are synthetic and natural antioxidants such as butylated hydroxyanisole (BHA) and polyphenols, respectively. However, because of the health concerns about synthetic antioxidants, natural alternatives have been getting more attention. It is also important to prevent food from any possible color, flavor and texture changes caused by antioxidants. In addition, being low cost, non-toxic, stable, and effective at low concentrations are desired properties of antioxidants (Kuai et al. 2021). The second part of an active packaging is the material. They can be produced by a renewable raw material, for example polysaccharides, such as chitin and starch, and proteins, such as gelatin and collagen. One of the most important features that distinguishes active packaging material from the traditional one is the potential of biodegradability. The meaning of biodegradability can be explained as the ability of decomposition by anaerobic digestion and then forming carbon dioxide, water, methane, and inorganic compounds. Biodegradation rate depends on some environmental factors such as humidity, temperature, type, and number of microorganisms (Said et al. 2023).

Active packages can be formed by fibers, which have high loading capacity and large surface area. There are several methods for fiber production such as blow spinning, electrospinning, and centrifugal spinning (Shen et al. 2023). Addition of some active agents to fibers create various types of active packaging systems such as antimicrobial, oxygen scavenging and antioxidant. Antimicrobial packaging aims to prevent the growth of microorganisms by containing natural or synthetic antimicrobial agents. Natural antimicrobial agents such as essential oils, capsaicin, and curcumin draw more attention as compared to the synthetic antimicrobial agents such as Zn, Cu and Ag nanoparticles, because of the safety and toxicity concerns (Shen et al., 2023). Still, there are some studies done with mentioned metal nanoparticles. For example, nylon-6 (Pant et al., 2012) and polyvinyl alcohol (Zhang et al., 2016) fibers with Ag nanoparticles were developed by electrospinning and the resulting fibers were shown to inhibit the microbial growth of *E. coli* and *S. aureus*. Natural active agents such as red propolis ethanolic extract enhanced with cloves and basil essential oils were incorporated to the gelatin-based films and showed an antimicrobial activity against

S. aureus and *S. Enteritidis* (Reyes et al., 2021).

Another study successfully showed the antimicrobial activity of eugenol on *E. coli* and *S. aureus* by producing poly (lactic acid) and gelatin-based electrospun fibers (Li et al., 2021). Bay laurel leaf essential oil is known with its antimicrobial activity as a natural antimicrobial agent, and it is effective on inhibiting microbial growth. According to the study about addition of bay laurel leaf essential oil to maize starch and rice protein films, the growth of *Bacillus cereus* bacteria was reduced during the storage of strawberries (Kurtfaki & Yildirim-Yalcin, 2023). Further information about bay laurel leaf essential oil will be given in upcoming sections.

Oxygen scavenging packaging aims to eliminate the excess oxygen in food packages since most of the food deterioration such as nutritional losses, color and sensory changes caused by oxygen. In order to solve this issue, oxygen scavengers such as ascorbic acid, and iron (Fe) can be used (Yildirim et al., 2018). In the literature, the study of Matche et al. (2011) aimed to develop oxygen scavenging food packages such as linear low-density polyethylene (LDPE) film added with ascorbic acid, zinc and iron showed an extended shelf life of bun and bread.

Antioxidant packaging systems also struggle with oxygen in food packaging, as similar with oxygen scavenging packages. However, oxygen scavengers work as an absorber, whereas antioxidants protect the food by releasing their active agent. In this aspect, synthetic and natural antioxidants take place to prevent the effects of oxygen in food packaging. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are two common synthetic antioxidants which have been used for a long time. However, concerns of chemical usage in food packaging arise natural antioxidants such as essential oils, plant extracts, and polyphenols to be used with the same purpose. Besides, by-products can also be used in active packages (Yildirim et al., 2018). For example, beet root residue powder (Iahnke et al., 2016) and wine grape pomace (Stoll et al., 2016) were used to improve the oxidative stability of by reducing the peroxide value with gelatin capsule residue film and cassava starch film, respectively. Mango leaf extract was incorporated in chitosan film to reduce the oxidation of cashew nuts.

According to the result, it was found that presence of 5% mango leaf extract showed an 56% higher oxidation resistance as compared to the control (Rambabu et al., 2019). Similarly, gelatin/starch-based films with corn stigma extract reduced the lipid oxidation of chilled beef by 60% in comparison to the control film (Boeira et al., 2021). In another study, gallic acid loaded lentil flour-based active nanofibers were produced by electrospinnig as a packaging material to enhance the oxidative stability of walnuts. According to the results, package with active nanofibers lowered the lipid oxidation of walnuts significantly, as compared to control package without nanofibers (Aydogdu et al., 2019). Likewise, gallic acid incorporated pea flour-based active films were produced by casting method to analyze their effect on lipid oxidation of olive oil. Results showed that gallic acid added films diminished the primary lipid oxidation products and total oxidation values (TOTOX) up to 28% and 20%, respectively, in comparison to control films (Yildiz, Bayram, et al., 2021).

Caffeic acid is also known as a natural antioxidant against lipid oxidation. İlyasoğlu and Guo (2019) synthesized a water-soluble chitosan-caffeic acid conjugate and formulated an emulsion using this conjugate. Their observations indicated a notable reduction in lipid oxidation in fish oil due to caffeic acid compared to chitosan. Detailed information about caffeic acid will be given in upcoming sections.

1.2. Centrifugal Spinning

Centrifugal spinning has been used in the industry for a long while to produce fiberglass for filtration applications and thermal insulation. However, the usage of polymers in fiber production by centrifugal spinning has been seen since 1990s. After that, large-scale centrifugal spinning devices were developed for nanofiber production from polymers. The patented technology, Forcespinning®, can produce nanofibers from different polymers by using rotating spinning heads with high speed. Centrifugal spinning method is a safer, cheaper, and faster way for fiber formation, and it also enables the use of various polymer solutions. Fiber formation by centrifugal spinning depends mainly on centrifugal force and surface tension of the polymer solution (Zhang & Lu, 2014).

1.2.1. Process and working principle

There are two main parts in the centrifugal spinning system: the rotating spinning head and the fiber collecting part. The polymer solution is put into an injector, which is on top of the spin device and connected to the spinning head with a pipe, then it is pushed to fill into the head. The polymer solution goes to the head, which has symmetrically placed nozzles on it, and then it produces fibers by ejecting the polymer solution from nozzles. The radius range of spinning head for the lab-scale centrifugal spinning devices is between 20 and 40 mm. It is easier to reach a rotation stability with a spinning head having smaller radius, especially at higher rotational speeds. The rotational speed of the head is adjustable and affects the diameter of fibers by altering the centrifugal force (Xu et al. 2023).

The second part of the system, the collector, is a cylinder that is located under the rotating spinning head, and it collects produced fibers on it. It rotates during the process and vacuum placed inside the cylinder helps to collect fiber without spreading them around. The distance between the collecting part and the spinning part is an important parameter in terms of the fiber morphology. If the distance is short, the time required to evaporate the solvent will be insufficient, therefore bead formation can be observed. In addition, collector might create airflow inside the spinning device and change the morphology of fibers due to the air friction caused by airflow (Chen et al. 2019).

To produce fibers with this method, centrifugal force and surface tension are important. Centrifugal force is formed due to the high rotation speed of the spinning head. Created force helps the polymer solution while it arrives to the orifice by pushing. If the centrifugal force overcomes the surface tension and the viscosity of the solution, fiber formation begins by evaporating the solvent. On the other hand, the surface tension is a parameter that depends on the polymer type and some properties such as solution concentration and viscosity might affect it. It is desired to lower the surface tension to form the fibers by overcoming the centrifugal force (Gholipour-Kanani and Daneshi 2022).

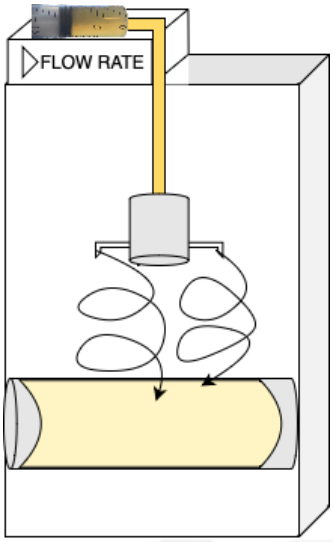


Figure 1.1 Schematic of centrifugal spinning device (Drawn by Draw.io)

1.2.2. Parameters affecting centrifugal spinning

The parameters that might affect the fibers produced by centrifugal spinning technique can be divided into two groups: Internal factors and external factors. The concentration of the polymer solution, the polymer and the solvent type are considered as internal factors that can affect the fiber formation since they change the viscosity and surface tension. On the other hand, external factors can be classified as spinning device parameters and environmental parameters. Spinning device parameters such as rotation speed of the spinning head, flow rate of the polymer solution, and diameter of the nozzle can be adjusted according to the type of the equipment. Environmental conditions such as temperature, and air humidity are defined also as external factors.

Among internal factors, polymer type and the concentration of the polymer solution are the main parameters responsible for the formation of chain entanglements. If the solution concentration is lower than the critical value, fiber formation becomes harder because of the unstable chain entanglement. In other words, intertwined or overlapped chains, which is the primary condition for the fiber formation, cannot be formed. Concentration also affects the viscosity and surface tension of the polymer solution. There are some polymers commonly used in centrifugal spinning studies such as chitosan, polylactic acid (PLA), polystyrene (PS), polycaprolactone (PCL), and their blends.

Viscosity of the polymer solution must be neither low nor high because in both cases fiber formation could be difficult. At lower viscosity, bead formation and splash of the polymer solution may be observed. On the other hand, at higher viscosity, it might be difficult to push the solution to the nozzles. Considering that the viscosity is adjusted to a range that polymer solution forms fiber, in this situation diameter of fibers enlarge with the increasing viscosity. In the study that developed polycaprolactone and gelatin-based fibrous scaffolds by centrifugal spinning, decrement in the viscosity of solution by changing the polymer blend ratios, created finer fibers (Loordhuswamy et al. 2014). Surface tension is also a critical parameter since fiber formation starts when centrifugal force overcomes surface tension. Bead or droplets might be observed in polymer solutions with high surface tension (Zannini Luz and Loureiro dos Santos 2022).

Solvent type selection is limited due to the necessity of complete solubilization. It also affects the viscosity and the surface tension of the polymer solution. Since solvent is evaporated during the spinning process, evaporation rate is important. Faster evaporation, in solvents with high vapor pressure, may cause fibers with porous structure and larger diameter, which can explain the prevention of polymer elongation (Zannini Luz and Loureiro dos Santos 2022).

External factors such as rotational speed of the spinning head, flow rate of the polymer solution and diameter of the nozzle can be adjusted according to the device parameters. Rotational speed of the spinning head is an important parameter since it creates centrifugal force, which is the primary factor to form fibers by exceeding the surface tension. Thinner fibers can be formed with high centrifugal force caused by higher the rotating speed. Lowering the rotational speed decreases the centrifugal force, however the surface tension does not change. This situation might lead to an increase in the bead formation. As an example, in the study of Lu et al. (2013), the effect of rotational speed on average fiber diameter was examined in centrifugally spun polyacrylonitrile nanofibers. At rotational speeds of 2000, 3000 and 4000 rpm, the average fiber diameter was found as 663, 541 and 440 nm, respectively. Similarly, the study of polyamide 6 nanofibers produced by centrifugal spinning showed that the increasing the rotational speed from 4000 to 9000 rpm reduced the average fiber diameter from 0.224 to 0.188 μm at the polymer concentration of 15% (Hammami et al., 2014).

Flow rate of the polymer solution affects the solvent evaporation; therefore, it determines not only the fiber diameter, but also the homogeneity of structure. Solution with higher flow rate enables solvent to evaporate quickly, as a result the diameter of fibers can get thinner. Conversely, the slower flow rate of solution extends the evaporation period and wet remaining fibers might be formed (Marjuban et al. 2023). Diameter of nozzle is another factor that influences the fiber formation. It controls the amount of polymer solution to be ejected from the tip of the nozzle. Nozzles with smaller diameter restricts the amount of solution and resulted in thin fibers. If the nozzle diameter gets very smaller, it may not be suitable to use them in centrifugal spinning because ejecting the solution becomes almost impossible.

Environmental factors such as temperature and air humidity may affect the evaporation of solvent. Temperature can influence the viscosity of the solution, in other words, at higher temperatures polymer solution remains melted for longer time and it affects the elongation of fibers. In the study of Shanmuganathan et al. (2012), increasing the temperature of polymer solution from 280 to 300 °C led to a decrement in the average fiber diameter from 1.64 to 1.17 μm. Other than temperature, humidity adjustment is important not only for the spinning period, but also during storage of fibers. It must be lowered in both cases, to obtain homogenous fibers and maintain their structure (Chen et al. 2019; Marjuban et al. 2023).

1.2.3. Differences between electrospinning and centrifugal spinning

Electrospinning is a fiber production method that has been widely used in different fields such as food packaging, sensors, textile, and medicine. It is made of three sections: spinneret part, high voltage power supply and collector. The first part, spinneret, stores the polymer solution to be spinned and helps to eject the solution from the syringe with needles by an injection pump. High voltage power supply part can provide alternating or direct current to allow the fiber formation with electric field. At the end of the process, formed fibers are collected on the collector. Different from the centrifugal spinning, electrospinning is based on the electrostatic repulsion created mainly by two electrodes. One of them is attached to the solution to increase the electrostatic potential of it and the other one is put to the collector. The reason for

applying electric field to the solution is to move the ions and to charge the surface of the solution. Created electrostatic force between the electrodes helps to overcome the surface tension, thus fibers are formed. In centrifugal spinning, the force that is desired to overcome the surface tension is centrifugal force (Valizadeh and Farkhani 2014). Although the logic behind the fiber formation is evaporating the solvent for both methods, requirement of high voltage supply makes electrospinning more difficult.

The parameters that affect electrospinning method are similar to those affecting centrifugal spinning such as viscosity and surface tension of the solution, polymer and solvent type, flow rate of the solution, and nozzle diameter. However, solution conductivity and applied voltage are important factors only considered in electrospinning (Li et al., 2021).

Compared to the electrospinning, centrifugal spinning offers safer and simple fiber production method due to the lack of high voltage requirement. Moreover, the solution conductivity is not a limitation for centrifugal spinning since electric field is not required, therefore it allows more polymer and solvent choices to be used. When these factors are taken into account, the production rate of lab-scale electrospinning process is almost 100 times slower than lab-scale centrifugal spinning device. This finding points out the efficiency and the high production rate of centrifugal spinning method. In addition, the faster production enables to lower the production cost (Atıcı et al., 2022). In a study by Yang and Yeum (2017), the morphological analysis of poly(vinyl alcohol) fibers produced by electrospinning and centrifugal spinning methods were compared. According to results, nanofibers had similar average diameter, alignment and melting temperatures. However, it was concluded that to produce large scales, centrifugal spinning can be preferred due to its high efficiency and low cost (Yang & Yeum, 2017). Another research compared the morphology of electrospun and centrifugal spun poly(vinyl alcohol) and poly(vinyl pyrrolidone) fibers. Although the same increasing trend of average fiber diameter was observed in higher polymer concentrations, larger diameters were obtained by centrifugal spinning. Moreover, it was stated that centrifugal spinning could offer technological advantages and quantitative production with similar fiber diameters (Rihova et al., 2020).

1.2.4. Studies on centrifugal spinning

The usage of centrifugal spinning and electrospinning is very similar, therefore same application areas are found such as biomedical applications, air filtration, and food packaging.

In biomedical field, centrifugal spinning has been used in tissue engineering, wound dressing, and drug delivery (Chen et al., 2019). In the literature, fibrous scaffolds for tissue regeneration (Loordhuswamy et al. 2014) and antimicrobial wound dressing (Xia et al. 2019) were the hot topics that centrifugal spinning studies focused.

Cremar et al., (2018) showed that chitosan-based fiber scaffolds produced by centrifugal spinning provided a potential wound dressing with its antimicrobial activity. Similarly, according to another study done by Gungor et al. (2021), $AgNO_3$ added gelatin based centrifugal spun biomats provided a wound dressing with its antibacterial activity. In another study, to reduce the environmental pollution, instead of single use plastic mask, N95 mask with gelatin nanofibers were obtained at a great filtration efficiency (Arican et al.2022), Another study focused on production of antibacterial gelatin-based fibers for tissue engineering (Akhtar et al. 2022).

Other than these fields, centrifugal spun fibers can be used for air filtration applications. Thermoplastic urethane was used to produce nanofibrous webs by centrifugal spinning as an air filter media. Optimization study was conducted with the parameters of polymer concentration, rotational speed and nozzle orifice diameter. It was found that the polymer concentration affected mostly the fiber diameter. The optimized fibers showed a potential for air filtration applications with 99.4% filtration efficiency (Gundogdu et al., 2018). Similarly, Tepekiran et al. (2019) successfully developed silica-based nanofibrous filters by centrifugal spinning to be used in industrial hot air filtration applications. In another study, dust and virus protected masks were developed with polystyrene, poly(methyl methacrylate), and polyvinylpyrrolidone by centrifugal spinning. Having up to 97% capturing efficiency, they exhibited potential textile material for future applications (Kwak et al., 2021).

In addition to these researches, there are also some studies in food industry to produce active food packaging. One of them is developing gelatin-based centrifugally spun fibers incorporated with lemon peel essential oil (Doğan et al., 2022b). In this study,

centrifugal spin solutions were prepared by dissolving 15% gelatin in acetic acid and adding different concentrations (1, 3.5 and 5%) of lemon peel essential oil. Flow rate and nozzle rotation speed were adjusted as 30 mL/h and 8000 rpm, respectively. In this study, homogenous fiber structure was obtained successfully, and the microbial growth was reduced. In addition, the shelf life of cheese was extended by using lemon peel essential oil added centrifugally spun fibers.

The other food related study was lavender essential oil containing polyvinylpyrrolidone (PVP) based centrifugally spun fibers (Doğan et al., 2022a). The solutions were prepared by dissolving PVP in ethanol at 10% concentration and lavender essential oil with the concentrations of 1, 3.5 and 7% were added. Flow rate and nozzle rotation speed were 30 mL/h and 8000 rpm, respectively. The produced fibers prevented the growth of psychotropic bacteria, aerobic mesophilic bacteria, yeast, and mold in minced meat. These two studies can be promising for the usage of active food packaging produced by centrifugal spinning.

1.3. Gelatin as a polymer in food packaging

One of the animal-based biopolymers, gelatin, is a biodegradable, renewable, low cost and abundant protein obtained by denaturation of collagen. It is almost odorless and tasteless material found in powder form. Many kinds of gelatin can be obtained from bovine, porcine, fish, insect, and poultry. Gelatin quality can be determined with the gel strength, in other words “bloom” value, which indicates the stiffness of the gelatin. Generally, bloom value range is between 30 and 300 bloom, reflecting the average molecular weight. Bloom values can be classified as low, medium and high, according to the range lower than 150, between 150-200, and higher than 220, respectively. As the bloom value increases, strength of gelatin enhances. Bloom value also affects the viscosity of gelatin, which is an important parameter for fiber formation. Viscosity may change at higher temperatures due to the denaturation possibility of three-dimensional gelatin structure, resulted in lower viscosity (Said et al., 2023). Gelatin is used not only as an ingredient in food products, but also as a coating material in capsules and packaging films. It is a preferable material for encapsulation studies due to its film forming property (Baranauskaite et al., 2019; Roy & Rhim, 2020).

These features enhance the application area of gelatin from cosmetic to biomedical, filtration and pharmaceutical industry (Alipal et al. 2019). Since one of the reasons for manufacturing of hard and soft drug capsules from gelatin is to protect active agent from atmospheric oxygen (Gómez-Mascaraque et al. 2015), it might be a good choice to encapsulate active compounds with gelatin for food packaging applications. Therefore, gelatin is one of the high potential candidates for an eco-friendly packaging material. There are various studies which uses gelatin in active packaging area. In one of the studies, date fruit waste was incorporated into gelatin film to impede lipid oxidation (Rangaraj et al. 2021). In another research, it was found that copper sulfide nanoparticles added gelatin films had an antimicrobial activity against *E. coli* and *L. monocytogenes* (Roy and Rhim 2021). It was possible to obtain an antimicrobial nano film with addition of 3-phenylacetic acid to gelatin (Liu et al. 2021). Fabrication of gelatin-based curcumin loaded active nanofibers were done by electrospinning with enhanced antioxidant activity, water vapor permeability and thermal properties (Hasan et al. 2023). In the study of Li et al. (2021), eugenol was loaded to gelatin-based electrospun nanofibers having antibacterial and antioxidant activity. It was shown that homogenous fiber structure with the average fiber diameter of 157 to 293 nm was obtained. Another study was conducted to produce gelatin-based nanofibers with the addition of thyme essential oil. Nanofibers showed great antimicrobial activity against *C. jejuni* on chicken and they did not exhibit any adverse effect on the color, textural, and sensorial properties of meat (Lin et al., 2018). Besides electrospinning, gelatin has been commonly used in centrifugal spinning studies. For instance, gelatin-based antibacterial wound dressing fibrous mats with the addition of silver nitrate were produced by centrifugal spinning. Fibrous mats were found as promising for wound dressing applications with their high air permeability (Gungor et al. 2021). Similarly, Loordhuswamy et al. (2014) developed polycaprolacrone and gelatin-based fibers as a wound dressing material. Fibers with smooth surface, suitable porosity and mechanical properties offered a suitable material for tissue engineering applications. However, there are not many gelatin-based centrifugally spun studies to be used as an active packaging material. Doğan et al. (2022b) conducted a study to produce gelatin-based centrifugally spun fibers incorporated with lemon peel essential

oil. Fibers, with bead free structure, exhibited antimicrobial activity against *E. coli* and *S. aureus,* indicating the potential of being active packaging material.

1.4. Caffeic acid as an active agent in food packaging

Caffeic acid, (3,4-dihydroxy cinnamic acid), is the foremost phenolic compound present in the legumes, olive oil, coffee, fruits, vegetables, and wine (Khan et al. 2021) . It has a variety of beneficial attributes, including antioxidant, antithrombogenic, anti-inflammatory, antibacterial, antiviral, antimutagenic, and anticancer activity (Ignatova et al. 2016). Some studies have revealed that consuming caffeic acid can decrease not only the risk of several cancer types, but also metastases in tumor cells (Alam et al., 2022). However, the main drawback of the caffeic acid is its low water solubility, chemical and physical instabilities which hinder its application. Moreover, its usage is limited due to being susceptible to environmental conditions. To overcome these problems and ease the usage by preserving its antioxidant activity, it can be encapsulated into the polymer network. Different encapsulation techniques such as electrospinning and electrospraying have been shown to be used (Khoshnoudi-Nia et al., 2020). These techniques not only enhance the delivery but also improves the mechanism of action (Khan et al. 2021; Pinho et al. 2014). Thanks to the catechol group with an α, β-unsaturated carboxylic acid, it can easily interact with reactive oxygen species. Therefore, it can be incorporated into the packaging films to inhibit or delay the lipid oxidation (Yu et al. 2013).

Caffeic acid was encapsulated to gelatin-based films from horse mackerel scales by casting method. The outcome of the study was not only enhanced antioxidant activity and total phenolic content, but also improved physical properties of films (Le et al., 2018). Another method, electrospinning, has already been used to encapsulate caffeic acid. Zeren and her colleagues (2022) developed caffeic acid loaded electrospun fibers from carob bean flour and whey protein polymer matrix. Results showed that encapsulation of caffeic acid increased the antioxidant activity of fibers and created a promising active packaging material (Zeren et al., 2022).

Other than this, poly(3-hydroxybutyrate)/poly(ethylene glycol) based fibers incorporated with caffeic acid were produced by electrospinning. Fibers with caffeic

acid showed an antibacterial activity to be used as a wound dressing (Ignatova et al. 2016).

Although there are few studies on encapsulation of caffeic acid by electrospinning, there is no study on the usage of centrifugal spinning on encapsulation of any phenolic acid. The disadvantages of electrospinning such as high voltage usage, low production rate, and limited appropriate solvent types can be overcome by centrifugal spinning, which is a novel technology (Zhiming and Jun. 2017).

1.5. Bay laurel leaf essential oil as an active agent in food packaging

Essential oils consist of volatile compounds extracted from some parts of plants such as leaves, flowers, seeds, and fruits. Nearly 3000 types of essential oil are found to be used as flavor or fragrance. In addition to that, they have antibacterial, antioxidant, and antiviral properties. Essential oils can be extracted by steam distillation or using hexane. Hexane extraction resulted in higher antimicrobial activity in comparison to steam distillation. To have highest antimicrobial activity, it is suggested to extract herbs during or directly after flowering of plants. The mechanism behind the antimicrobial activity of essential oils based on their hydrophobicity, which allows to destroy the cell membrane by making it permeable. This destruction resulted in the leakage of components inside the cell, and finally lysis occurs. Antimicrobial potential of essential oils can be used in food packaging systems to prevent the microbial growth and control the contamination. However, addition of essential oil directly to foods may be resulted in taste and sensorial changes. Therefore, incorporation of essential oils into the packaging material is a promising method (Vergis et al., 2015).

One of the essential oil types produced from Bay laurel leaf (*Laurus nobilis* L.), is a part of Mediterranean plant, which is known not only as a flavor, but also a traditional healer (Dobroslavić et al. 2023). It is used for the treatment of respiratory problems, diabetes, inflammation, cancer, and fungal infections. Besides, it has a positive effect on skin health, especially wound healing (Šunić et al. 2024). By containing high amount of polyunsaturated fatty acids and omega-3 fatty acids, it has a desirable aspect for human diet (Dobroslavić et al. 2022). Essential oil from laurel plant has an antioxidant, antimicrobial and anti-inflammatory effect, which is commonly used in cosmetic sectors (Kendir et al. 2024).

In addition to the usage in cosmetic sector, these effects might be promising in active packaging systems (Devi et al. 2024). Bay laurel leaf essential oil has ability to act as an antimicrobial agent by modify cell membrane permeability and integrity of bacteria because of its volatile compounds such as terpenes, terpenoids and phenols (Göksen et al. 2020). Due to its essential oil, flavanol, flavones and phenolic acids, bay laurel leaf has high polyphenol content. However, environmental conditions such as light, heat, humidity and pH pose a threat for polyphenols to degrade. Therefore, it is important to use encapsulation methods to make them more stable (Dobroslavić et al. 2023).

Up to now, numerous studies have been conducted to encapsulate laurel essential oil with different techniques. For example, maize starch and rice protein based edible films were developed by drying film-forming solutions. According to results, strawberry samples with film showed enhanced shelf life and visual quality during five days storage (Kurtfaki and Yildirim-Yalcin 2023). Another study was done microencapsulation by spray drying. This method enabled to improve the bioaccessibility of laurel leaf polyphenols (Dobroslavić et al. 2023). Electrospinning technique was also studied to encapsulate laurel leaf essential oil with zein fibers. Produced fibers showed homogenous structure and reduced the microbial growth of *L. monocytogenes* and *S. aureus.* In addition, the inhibition of aerobic mesophilic bacteria was observed in the cheese samples coated with film (Göksen et al. 2020).

1.6. Objectives and novelty of the study

One of the fiber producing method, electrospinning, has been used for a long time to produce fibers with active agents. Although there are various studies done by electrospinning, its drawbacks emerged a new method named as centrifugal spinning. With this method, fibers can be produced as in electrospinning by eliminating its high voltage requirement. Therefore, centrifugal spinning offers safer and cheaper method to produce fibers in a shorter time with high efficiency. In addition, spinnability of polymer type is wider in comparison to other fiber producing techniques. Produced fibers by centrifugal spinning can be used for active food packaging.

Gelatin is one of the suitable animal-based polymer type to be centrifugally spinned, that is also used in food industry.

It is not only a food ingredient, but also a fiber forming material by being nontoxic, low cost, biodegradable and abundant. It can cover the active agents that incorporated inside successfully.

In the literature, there is gap about producing gelatin-based fibers to be used as an active food packaging by centrifugal spinning method. In addition, there is no study that develops gelatin fibers incorporated with caffeic acid and bay laurel leaf essential oil (BLLEO). Moreover, the effect of caffeic acid and BLLEO concentration on oxidation of olive oil has not been studied yet.

This study aimed to generate biodegradable and environmentally friendly gelatin-based active fibers containing caffeic acid and BLLEO through centrifugal spinning and to characterize the resulting films across varying concentrations of active agents. Moreover, BLLEO added samples were optimized according to three different parameters: Flow rate of the spinning solution, concentrations of gelatin and BLLEO to select thinner average fiber diameters with the highest total phenolic content and antioxidant activity. Produced active fibers were characterized in terms of morphology, antioxidant activity, total phenolic content, encapsulation efficiency, water vapor permeability, lipid oxidation, thermal analysis, Fourier Transform Infrared Spectroscopy (FTIR), biodegradability and antimicrobial analysis. In addition, it was aimed to assess the effectiveness of active fibers as packaging materials in terms of reducing the oxidation rate of olive oil. Both primary (peroxides) and secondary oxidation products (Thiobarbituric acid reactive species – TBARS) along with aldehydes such as 2-alkenals and 2,4-dien-als were measured to evaluate this effect.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Bovine gelatin, which had a Bloom value of 200, was provided by Eti Food Industry Co. Inc. (Eskisehir, Türkiye). Bay laurel leaf essential oil was provided by Arifoglu Biomedical Cosmetics Food Co. Inc. (Istanbul, Türkiye). Acetic acid, caffeic acid and 2,2-diphenyl-1-picrylhydrazy were purchased from Sigma-Aldrich ChemieGmbh (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent, gallic acid, ethanol, methanol, sodium carbonate, Tween 80 were purchased from Merck (EMSURE; ACS, Reag. Ph Eur., Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of centrifugal spinning solutions

2.2.1.1. Gelatin/Caffeic acid solution

Gelatin solutions with the concentration of 25% were prepared by dissolving gelatin in acetic acid. In order to obtain a homogenous solution, they were mixed at 700 rpm at 40°C overnight, by a magnetic stirrer (MaxTir 500, Daihan Scientific, Seoul, Kore). Different concentrations of caffeic acid were dissolved in ethanol: water (80:20 v/v) 2% and 3% (w/w) based on gelatin, which were decided according to the maximum solubility of caffeic acid in 80% ethanol. Solution with no caffeic acid was also prepared as control. Then, to decrease the surface tension and to obtain homogenous fiber morphology, Tween80 was added to the gelatin solutions as a surfactant (0.8% (w/v)) (Zeren et al., 2022). Control (0%), 2% and 3% of caffeic acid added fibers were named as G-C, G-2CA and G-3CA, respectively.

2.2.1.2. Gelatin/Bay laurel leaf essential oil solution

The full factorial design was used to establish a second-order (quadratic) model to

explore the impact of flow rate (X_1) at three levels (10, 15, and 20 mL/h), gelatin concentration (X_2) at two levels (20 and 25%), and essential oil concentration (X_3) at three levels (1, 3, and 5%) on response variables, including fiber diameter (Y_1), total phenolic content (Y_2), and antioxidant activity (Y_3) of the fibers for this part of the study. However, it was not possible to spin the solution at a gelatin concentration of 25% and a flow rate of 10 mL/h. Thus, the total number of experimental runs was reduced from 18 to 15 experiments (Table 2.1). Multiple Regression Analysis tool in MINITAB® Release 19 (Minitab Inc., State College, PA, USA) was used for analyzing main effects and interactions, and regression optimization tool for optimization purpose. After the optimization process, four different nanofibers were investigated for further analysis prepared at optimal gelatin concentration and flow rate, incorporating 1%, 3%, and 5% BLLEO to observe the effect of BLLEO, alongside control fibers containing no BLLEO (0%). These fibers were assigned as BLLEO-1, BLLEO-3, and BLLEO-5.

According to the design of experiments, gelatin solutions with the concentration of 20% and 25% were prepared for fiber formation by first dissolving gelatin in acetic acid. The solution was thoroughly mixed using a magnetic stirrer (MaxTir 500, Daihan Scientific, Seoul, Korea) overnight at 700 rpm and 40°C. Then, BLLEO was added to the prepared gelatin solutions at the concentrations of 1%, 3% and 5% (v/v). To achieve homogeneous fiber structure and reduce surface tension, 0.8% (w/v) of Tween80 was added as a surfactant to the gelatin solutions. Fibers with 1%, 3% and 5% of BLLEO were named as BLLEO-1, BLLEO-3 and BLLEO-5, respectively.

Table 2.1 Experimental data in regression design

Run	*Factor 1* X_1: *Flow rate (mL/h)*	*Factor 2* X_2: *Gelatin concentration (%)*	*Factor 3* X_3: *BLLEO concentration (%)*
1	15	20	1
2	15	20	3
3	15	20	5
4	10	20	1
5	10	20	3
6	10	20	5
7	20	20	1
8	20	20	3
9	20	20	5
10	15	25	1
11	15	25	3
12	15	25	5
13	20	25	1
14	20	25	3
15	20	25	5

2.2.2. Rheological properties of solutions

Rheological properties of solutions were determined by using a controlled strain rheometer (Kinexus dynamic rheometer, Malvern, Worcestershire, UK) which has cone with 4° cone angle and plate with 40 mm diameter at 25 °C. For the measurement, prepared solutions were placed carefully, and the edges were trimmed if necessary. Shear stress values were recorded between shear rates of 0.1 and 100 s^{-1} (Yildiz et al., 2022).

2.2.3. Centrifugal spinning of solutions

To fabricate the fibers, the method described by Doğan et al. (2022b) was followed with some modifications. Fibers were fabricated at the room temperature (25°C) by using Nanocentrino L1.0TM (Areka Group LLC, Istanbul, Türkiye). Centrifugal spinning conditions were determined according to preliminary experiments.

The volumetric flow rate of caffeic acid fibers was set as 10 mL/h, whereas BLLEO loaded fibers was 10, 15 and 20 mL/h. Spindle speed were adjusted as 1800 rpm for both active ingredients. Two needles were used with 23 G (Gauge) diameter by attaching them to the part of the device. Equal amount of solution (20 mL) was put into a syringe for each turn. Nonwoven polyester spunbond was put onto the vacuum collector in order to collect fibers. The speed of the vacuum collection portion is adjusted at 600 rpm.

2.2.4. Characterization of fibers

Fibers were analyzed in terms of morphology, total phenolic content (TPC), antioxidant activity (AOA), encapsulation efficiency (EE), water vapor permeability (WVP), Fourier Transform Infrared analysis (FTIR), thermal analysis, lipid oxidation in olive oil, antimicrobial activity, and biodegradability.

2.2.4.1. Morphological analysis

The morphological analysis of films was done by Field Emission Scanning Electron Microscopy (FESEM) (JEOL, Tokyo, Japan) at 2000 magnification. Diameters of fibers were measured manually by ImageJ (Maryland, USA). 100 random measurements were done with different images and average diameters with distributions of them were determined (Yildiz, Sumnu, et al., 2021).

2.2.4.2. Total Phenolic Content (TPC)

Total phenolic content was measured by Folin-Ciocalteu method given in Luca et al. (2013) with some modifications. For caffeic acid fibers and BLLEO fibers, sample (0.03 g) was mixed with 15 mL and 5 mL ethanol: water (80:20 v/v) solution, respectively. After that, high speed homogenizer (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH&Co. KG, Staufen, Germany) was used for 1 min at 3000 rpm in order to obtain phenolic extracts. Solution was filtered by using chromatography syringe filter (ISOLAB - PTFE - 45/25) and 0.5 mL of diluted sample was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu solution and vortexed (ZX3; VELP Scientifica, Usmate, MB, Italy) for 1 min. Then, sample was put into dark place for 5 min and then

2 mL of 7.5% (w/v) sodium carbonate solution was added. After keeping mixture in the dark for 1 h, absorbance of samples was measured at 760 nm by using UV/VIS spectrophotometer (UV 2450, Shimadzu, Columbia, USA).

For the calibration curve, gallic acid was dissolved in ethanol: water (80:20 v/v) solution with different concentrations (10, 20, 40, 60, 80, 100 mg/L) and the same procedure was followed. Two replicates were done, and the results were determined as mg GAE (gallic acid equivalent) with the given Eq. (1),

$$\text{TPC (mg GAE/g film)} = \frac{C \times V \times D}{W_s} \quad (1)$$

where C, V, D and W_S are the concentration of the measured absorbance value from the calibration curve (mg/L), volume of the solution used for dissolving the film (L), dilution ratio and weight of the film (g), respectively.

2.2.4.3. Antioxidant Activity (AOA)

DPPH radical scavenging activity was determined by using the method given in Yildiz et al. (2022) with some changes. Fibers were dissolved in 10 mL of ethanol: water (80:20 v/v) solution. Then, prepared mixtures were centrifugated (MIKRO 220R Hettich Zentrifugen, Tuttlingen, Germany) at 5000 rpm for 10 min. After centrifugation, diluted sample solution and 25 ppm DPPH (2, 2-diphenyl-1-picrylhydrazyl) were mixed and vortexed for 1 min. Finally, after keeping samples in dark for half an hour, absorbance values were determined by using spectrophotometer (UV2450, Shimadzu, Columbia, USA) at 517 nm. Antioxidant activity was calculated from Eq. (2) below,

$$\text{Antioxidant Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

where, $A_{control}$ is the absorbance of the control sample and A_{sample} is the absorbance of the fibers.

2.2.4.4. Encapsulation Efficiency (EE) of fibers loaded with caffeic acid

EE values were determined by modifying the method of Yao et al. (2016). Theoretical TPC of gelatin fibers was measured by dissolving caffeic acid in 5 mL ethanol: water (80:20 v/v) solution and conducting the same procedure of the TPC analysis given in the "2.2.4.2. Total Phenolic Content (TPC)" section. For experimental phenolic content, total phenolic content results were considered. Final percentage values of EE were determined by using Eq. (3).

$$\text{EE}(\%) = \frac{Experimental\ phenolic\ content\ of\ the\ fiber}{Theoretical\ phenolic\ content\ of\ the\ fiber} \times 100 \qquad (3)$$

2.2.4.5. Encapsulation Efficiency (EE) of fibers loaded with BLLEO

To determine the EE values of fibers loaded with BLLEO, total grams of fibers formed during spinning process were measured by weighting the empty and the final nonwoven polyester spunbond at the beginning and at the end of the spin. Then, by considering collected fiber grams, the amount of TPC of samples were calculated and proportioned to the theoretical TPC of fiber emerging from the TPC of BLLEO (Eq (4)) measured as in section 2.2.4.2.

$$\text{EE}(\%) = \frac{Experimental\ phenolic\ content\ of\ the\ fiber}{Theoretical\ phenolic\ content\ of\ the\ fiber} \times 100 \qquad (4)$$

2.2.4.6. Water Vapor Permeability (WVP)

In order to perform WVP analysis, gravimetric method explained by Aydogdu et al. (2018) was used with some modifications. To obtain 100% relative humidity, 30 mL of distilled water was put into each cup, which had cylindrical shape with 40 mm internal diameter. After that, fibers were cut to suit the size of the cup and by using a digital micrometer (LYK 5202, Loyka, Ankara, Turkey) thickness of samples were measured from six different points. Then, samples were put on the cups and screwed. The rubber joint was placed between the cup and sample to prevent water vapor to permeate except fiber. Before putting cups into the desiccator, initial weight was

measured. Relative humidity of desiccator was set around 10% with the help of silica gels at the bottom of the desiccator. During 8 h, temperature and relative humidity were recorded by using humidity/temperature logger (EB120-TH1, EBRO, Ingolstadt, Germany), and weights of cups were recorded once an hour. Three replicates were done for each sample. Finally, water vapor permeability was determined with the help of Eq. (5) given below.

$$\text{WVP } (gm^{-1}s^{-1}Pa^{-1}) = \frac{G \times x}{t \times A \times S(R_1 - R_2)} \quad (5)$$

where, G, x, t, A, S, R_1, and R_2 represents water vapor flow in grams, fiber thickness (m), time in seconds, area of used sample (m^2), saturated water vapor pressure in Pascal at measured temperature, relative humidity inside the cups and relative humidity inside the desiccators, respectively.

2.2.4.7. Fourier Transform Infrared Analysis (FTIR)

In order to examine the chemical structure of fibers and to understand whether there was interaction among the components, FTIR analysis was performed. FTIR device (IR-Affinityl, Shimadzu, Kyoto, Japan) was used to analyze films with wavenumber between 400 and 4000 cm^{-1} by scanning 16 times. Two replicates were measured for each sample.

2.2.4.8. Thermal Analysis (TGA and DSC)

Thermogravimetric analyzer (Perkin Elmer Pyris 1, Perkin- Elmer Co., Norwalk, USA) was used for thermogravimetric analysis with about 5 mg of fiber samples and gelatin powder. They heated at a rate of 10 °C/min with a 30 mL/ min nitrogen flow rate from room temperature to 600 °C. Analyses were done in duplicate.

Differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, Massachusetts, USA) was used to analyze the thermal properties of BLLEO added fiber samples with nitrogen as a cooling system. Every sample, about 5 mg, was put in an aluminum pan

and heated from 25°C to 300°C at 5 ºC/min heating rate. For reference, an empty sample pan was used. For each sample, the analysis was done twice.

2.2.4.9. Lipid Oxidation

Four grams of refined olive oil were dispensed into petri dishes, subsequently supplemented with 0.3 g of fibers. Subsequently, the petri dishes were exposed to the atmosphere, left uncovered, and incubated in a dark oven (Nuve NO55, Ankara, Türkiye) at 40 °C for a period of 14 days. Peroxide values (POV), thiobarbituric acid reactive substances (TBARS), and *p*-anisidine values (PAV) were measured on days 1, 7, and 14 of storage with three replications.

Peroxide levels in samples were assessed following the method described by Yildiz, Bayram, et al. (2021) with minor changes. This procedure entailed placing 0.1 g of oil samples into a flask, followed by the addition of a 0.6 mL mixture of chloroform and acetic acid (in a 2:3 v/v ratio) to facilitate the dissolution of the oil samples via agitation. Subsequently, 0.01 mL of saturated potassium iodide was introduced into the solution, which was then agitated for 1 min. To this mixture, 0.6 mL of distilled water was added, followed by the inclusion of 0.02 mL of a starch solution (1 g/100 mL water). Then, the mixture underwent titration using a 0.005 N sodium thiosulfate solution until it turned transparent. The POV were then calculated using a specific equation (Eq. 6).

$$\text{POV (mEq peroxide/kg of oil)} = \frac{(S-B)\times N\times 100}{W} \quad (6)$$

where B, S, N, and W denote the blank titrant volume (mL), the sample's titrant volume (mL), the normality of the $Na_2S_2O_3$ solution, and the weight of the sample (g), respectively.

PAV determination process was conducted by adapting methods described by (Atencio et al., 2020) with some modifications. An oil sample weighing approximately 0.1 g (m) was dissolved in 5 mL of isooctane, and its absorbance was measured at

350 nm using a UV-VIS spectrophotometer (T80+, UV/Vis. spectrometer, PG Instrument Ltd., Lutterworth, UK), denoted as A_1. Subsequently, 1 mL of a p-anisidine solution (0.25%) was introduced into the solution and allowed to incubate in darkness for 10 min before re-measuring the absorbance at 350 nm, recorded as A_2. PAV was calculated using the formula described in Equation (7), employing isooctane as a substitute for the sample in the control (A_k).

$$\text{PAV} = \frac{25 \times [(1.2 \times (A_2 - A_k)) - A_1]}{m} \quad (7)$$

The evaluation of TBARS followed a procedure established in prior research (Yildiz, Bayram, et al., 2021). In this method, 20–80 mg of olive oil was weighed (m), and subsequent addition of 1-butanol continued until reaching a final volume of 5 mL. Moreover, 50 mg of 2-TBA reagent was dissolved in 25 mL of 1-butanol. Following this, 2 mL of the resultant solution was mixed in equal measure with the oil-butanol blend. The resulting combination underwent a 2-h heating process in a water bath adjusted to 95 °C. Post-cooling, absorbance was measured at 532 nm using a UV/VIS spectrophotometer (A_S). The TBARS content of the samples was expressed as mg malondialdehyde (MDA) per gram of oil, calculated using Equation (8) delineated below.

$$\text{TBARS} = \frac{50 \times (A_S - A_B)}{m} \quad (8)$$

The total oxidation (TOTOX) values for the oil samples were derived from the obtained POV and PAV data, following the method outlined by (Chong et al., 2015) through the application of the subsequent equation (9).

$$\text{TOTOX} = 2\text{POV} + \text{PAV} \quad (9)$$

2.2.4.10. Antimicrobial Activity

Antimicrobial activity was evaluated using the agar disc diffusion method by adapting the method given in Doğan et al. (2022b). Gram-positive bacteria *S. aureus* and

gram-negative bacteria *E. coli* were used to test the antibacterial properties of fibers against food-borne pathogens. Stock cultures were initially revived. To achieve this, cultures were put into 5 mL Mueller Hinton Broth (MHB) with a loopful of each microorganism, and the cultures were incubated for the entire night at 37 °C. Then, the cultures were diluted to 10^8 CFU/mL according on the 625 nm absorbance. A sterile swab was used to streak a Petri dish (Mueller Hinton Agar) after 100 µL of diluted bacterial cell solution was introduced. Fibers with 1 cm of diameter were cut. After setting the discs on the medium, petri dishes were incubated at 37 °C for 24 hours for the growth of *S. aureus* and *E. coli*. Then, the density of bacteria growing area around the discs in petri dishes was measured manually by ImageJ (Maryland, USA), and results were given as inhibition zone%.

2.2.4.11. Biodegradability of fibers

Biodegradability analysis of films was done with some modifications as given by (Zeren et al., 2022). Due to the soil remaining on the film surfaces, the weight changes would not be correct, therefore it was examined qualitatively. Soil was put into plastic cup (30 × 20 cm) with a height of 15 cm. Samples were cut as a square with dimensions of 2 cm × 2 cm and placed in a support. After that, prepared samples were buried into the soil and covered fully. In order to keep the soil moist, water was sprayed each day. Temperature and relative humidity adjusted as room temperature (22 ± 2 °C) and 65% ± 5%, respectively. Every day, samples were taken out from the soil and photographed, until degradation was completed.

2.2.5. Statistical Analysis

MINITAB (Version 16, State College, PA, USA) software was used for statistical analysis. Experimental results were evaluated by one-way analysis of variance (ANOVA). Tukey's multiple comparison test was done if there was significant difference ($p \leq 0.05$). Experiments were conducted in duplicate.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Fabrication of Gelatin/Caffeic acid fibers by centrifugal spinning

In the preliminary experiments, it was found that at gelatin concentrations below 20%, instead of fibers, only droplets were formed. Therefore, 25% gelatin concentration was selected to be used. The concentrations of caffeic acid, that will be used in the experiments, were determined according to the maximum solubility of caffeic acid in 80% ethanol.

3.1.1. Rheological properties of solutions

In centrifugal spinning process, viscosity is an important parameter for solutions since it affects the fiber formation. Viscosity must be neither too low nor too high, thus it must be controlled. For solutions with low viscosity, instead of fibers, formation of beads in SEM images or droplets during spinning process may be observed. Conversely, high viscosity may be a problem in terms of large gravitational force between molecules; therefore, it causes the molecules to become entangled. In this situation, the centrifugal force cannot remove the solution out; therefore, fibers could not be formed (Zhiming & Jun, 2017). Figure 3.1 shows the curves of shear stress versus shear rate for each sample solution. Newtonian behavior was observed in the samples with R^2 values of 0.999. According to Leuenberger (1991), gelatin solutions at 40°C showed Newtonian behavior at shear rate values between 10 and 350 s^{-1}. Since the solutions were prepared at 40°C and the shear rate values ranged between 0.1 and 100 s^{-1} for this study, it was not surprising to observe Newtonian behavior. Moreover, in another research about flow properties of gelatin solutions, similar result was obtained (Wulansaria et al., 1998). In this study, viscosity values were ranged between 0.261 ± 0.01 and 0.323 ± 0.01 Pa s. As can be seen in Table 3.1, there was no significant difference between viscosity values of the solutions. This result was expected because the only difference in each solution was the caffeic acid concentration, which was up to 3%.

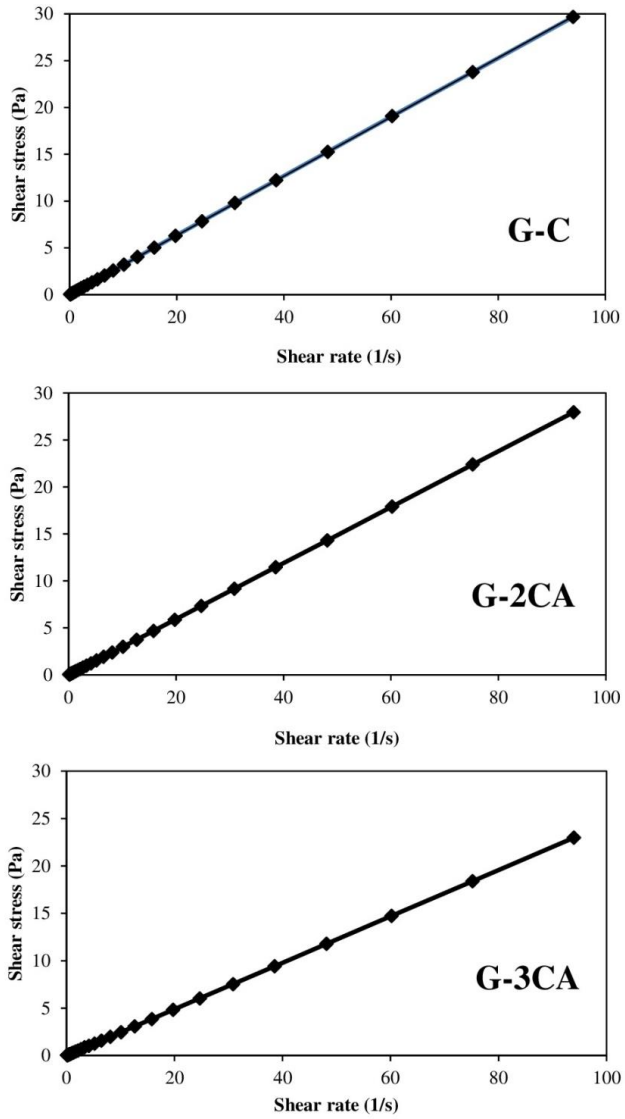


Figure 3.1 Shear stress versus shear rate curves of sample solutions.

Table 3.1 Viscosity, average diameter, and water vapor permeability of fibers.

Samples	*Viscosity (Pa.s)*	*Average Diameter (nm)*	*WVP* $\times 10^{-12}$ $(g.m^{-1}.s^{-1}.Pa^{-1})$
G-C	0.323 ± 0.01^{a}	1789.3 ± 538^{a}	2.77 ± 0.05^{b}
G-2CA	0.314 ± 0.03^{a}	1839.9 ± 502^{a}	4.32 ± 0.17^{a}
G-3CA	0.261 ± 0.01^{a}	1882.8 ± 499^{a}	4.13 ± 0.38^{a}

Columns with different letters are significantly different ($p \leq 0.05$)

3.1.2. Characterization of fibers

3.1.2.1. Fiber morphology

There are some other factors that affect the fiber morphology such as speed of the spinneret, distance of the collector and flow rate of the solution (C. Chen et al., 2019; Zhiming & Jun, 2017). Since the operation parameters were constant for all samples, the only parameter that might affect the morphology of fiber was the viscosity of the solution. The average fiber diameter values were given in Table 3.1 as 1789.3 ± 538, 1839.9 ± 502 and 1882.8 ± 499 nm for G-C, G-2CA and G-3CA samples, respectively. In a study, which developed centrifugally spun gelatin-based fibers with lemon peel oil, the average diameters of fibers were ranged between 1717.84 ± 38.21 and 2394.55 ± 44.25 nm (Doğan et al., 2022b). In this study, since there was no significant difference between viscosity values, the average diameters of fibers were found to be not significantly different. In another study that developed the cinnamon extract added chitosan/gelatin membrane by electrospinning, it was observed that the morphology of the fibers did not change as cinnamon extract concentration increased (Ahmadi et al., 2021). Diameter distributions and FESEM images of films were given in Figure 3.2. FESEM images showed that homogenous films were obtained without bead formation. Moreover, caffeic acid addition did not affect structure of fibers adversely. In previous studies, bead-free homogenous structures were also obtained in centrifugally spun gelatin-based films (Arican et al., 2022).

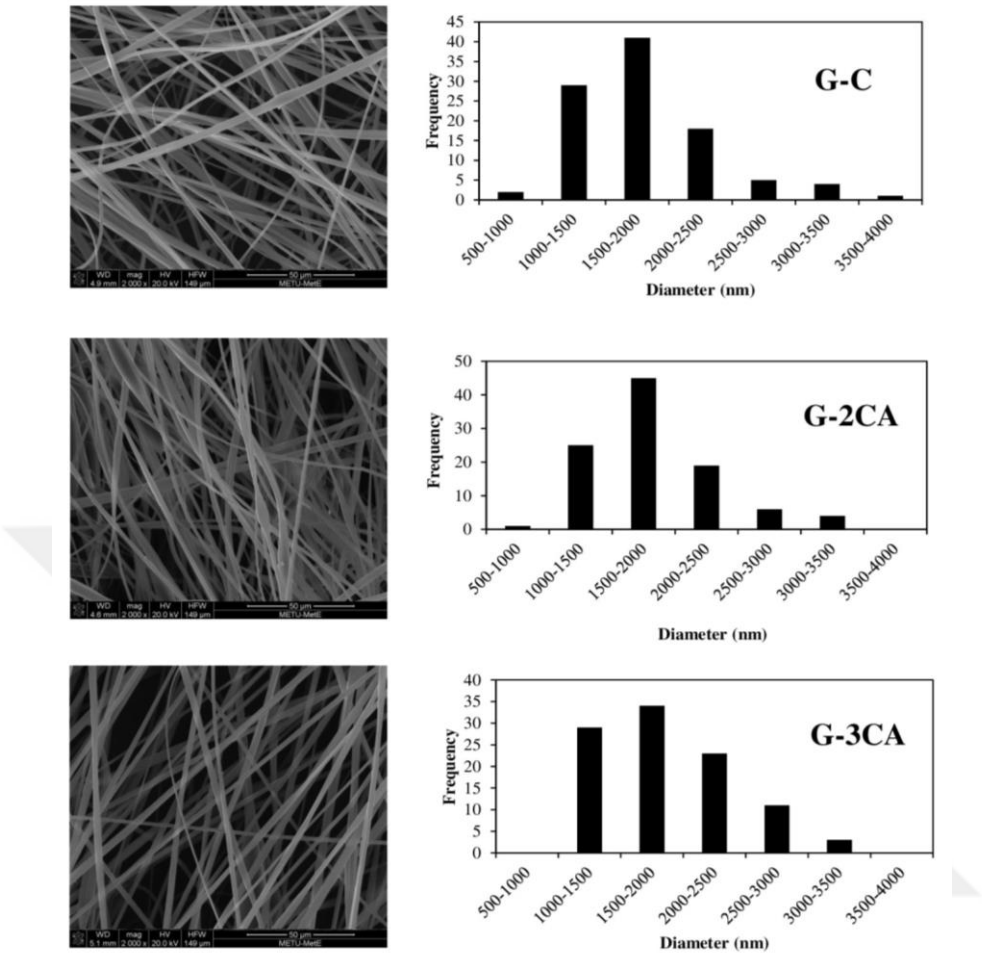


Figure 3.2 FESEM images and fiber size distribution of fibers.

3.1.2.2. Total Phenolic Content (TPC), Antioxidant Activity (AOA) and Encapsulation Efficiency (EE)

Total phenolic content analysis depends on the transfer of electron between Folin-Ciocalteu reagent and phenolic compound (Tavassoli-Kafrani et al., 2017). As can be seen in Table 3.2, there was a significant difference between total phenolic content of G-2CA and G-3CA, which were 21.31 ± 0.70 mg GAE/g and 26.88 ± 0.32 mg GAE/g, respectively. Fibers with higher caffeic acid concentration had higher TPC. Similar results were obtained in a study which investigates the influence of different phenolic compounds of gelatin film. Caffeic acid added films showed an increase of total phenolic content from 6.2 ± 0.1 mg GAE/g to 11.2 ± 0.3 mg GAE/g when the concentration of caffeic acid increased from 1% to 3% (Le et al., 2018). Since phenolic

compounds have an ability to play role in free radical reactions as an electron donor, they might be related to the antioxidant activity (Alparslan, 2018). Active agents with antioxidant activity are commonly used for active food packages since they can extend the shelf life of the food and can prevent from lipid oxidation (Zhang et al., 2020). In addition, the second hydroxyl group in ortho or para position could improve the antioxidant activity of caffeic acid because of the o-quinone or p-quinone formation and high resonance stabilization (Zeren et al., 2022). In Table 3.2, it can be seen that antioxidant activity of films increased as the caffeic acid concentration increased. Similar results were obtained in a study, which showed the potential of caffeic acid in terms of enhancing the antioxidant activity of films (Wang, Du, et al., 2019). For control fibers (G-C), both total phenolic content and antioxidant activity were not detected. Similarly, in a previous study about analysis of film forming gelatin solutions containing different essential oils the same result was observed (Alparslan, 2018). In this paper, it was found that antioxidant activity increased with the increasing total phenolic content. In other words, antioxidant activity followed a similar pattern with total phenolic content. Increasing caffeic acid concentration resulted in higher total phenolic content and antioxidant activity. This result showed the possibility of using these fibers as antioxidant active packaging material. As can be seen in Table 3.2, EE results were found as 95.70% ± 3.11% for G-2CA and 84.30% ± 3.25% for G-3CA, with a significant difference. The reason for decreasing EE with increasing caffeic acid concentration may be the precipitation of bigger crystals of caffeic acid in the syringe, that solution was put inside, during spinning process (Gómez-Mascaraque et al., 2017).

Table 3.2 Total phenolic content (TPC), antioxidant activity (AOA), encapsulation efficiency (EE) of fibers

Samples	*TPC (mg GAE/g)*	*AOA (%)*	*EE (%)*
G-C	N.D.	N.D.	N.D.
G-2CA	21.31 ± 0.70^{b}	72.00 ± 1.41^{b}	95.70 ± 3.11^{a}
G-3CA	26.88 ± 0.32^{a}	84.95 ± 1.06^{a}	84.30 ± 3.25^{b}

Columns with different letters are significantly different ($p \leq 0.05$).
N.D. means “Not Detected”.

3.1.2.3. Water Vapor Permeability (WVP)

Water vapor permeability is important in terms of improving packaging quality parameter since it controls transfer of water between the surrounding and food product. It may restrict the usage of food packaging if the WVP is insufficient (Ekramian et al., 2021). As it can be seen in Table 3.1, there was a significant difference between WVP values of G-C and caffeic acid added samples. In other words, as compared to control sample, the addition of caffeic acid led to an increase in WVP of the films. The reason for that may be the existence of polar groups in caffeic acid (Nuthong et al., 2009). The increase in the number of polymer's available polar (-OH) groups corresponds to an elevation in the WVP of the film (Moosavi et al., 2020). Similarly, in a study, in which caffeic acid was encapsulated in carob bean flour and whey protein-based nanofibers by electrospinning, the presence of caffeic acid caused an increase in WVP. Sample with 1% (w/ w) caffeic acid had $2.06 \pm 0.08 \times 10^{-10}$ g. $s^{-1}.m^{-1}$. Pa^{-1}, while sample without caffeic acid had $1.38 \pm 0.14 \times 10^{-10}$ g. s^{-1}. m^{-1}. Pa^{-1} WVP values (Zeren et al., 2022). On the other hand, there was no significant difference between G-2CA and G-3CA. Similar result was obtained in a previous research, which studied the effect of phenolic compounds on porcine plasma protein-based film. When different concentrations of caffeic acid (1%, 2% and 3%) were added to the films, there was no significant difference between WVP values (Nuthong et al., 2009).

3.1.2.4. Fourier Transform Infrared Analysis (FTIR)

In Fig. 3.3, FTIR spectra of samples are shown. G-C, G-2CA and G-3CA had similar peaks at 1636 cm^{-1} and 1522 cm^{-1}, which represents Amide I and Amide II band, respectively. In addition, there was also a peak around 600 cm^{-1} wavelengths, which corresponded to Amide III. Amide I, Amide II and Amide III regions indicating C−O stretching, N–H bending, and C–N stretching, respectively (Hassan et al., 2021). In similar research about electrospun gelatin nano- fibers with phenolic compounds, comparable FTIR ranges were reported for gelatin with caffeic acid (Tavassoli-Kafrani et al., 2017). On the other hand, for pure caffeic acid FTIR spectra, peaks can be observed frequently between the range of 500 and 1500 cm^{-1}. The reason for

frequencies lower than 1120 cm^{-1} might be the bending of C–C–C for aromatic system. Another aromatic bending corresponded to the peaks around 1217 cm^{-1}. Moreover, two peaks can be seen at around 3200 and 3400 cm^{-1}, which indicated –OH stretching vibration (Vertuccio et al., 2023). The two peaks, which correspond to Amide I and Amide II band, were seen in all three samples. These bands were overlapped with the characteristic peaks of the pure caffeic acid. When the peak intensities were compared, increasing trend can be seen from G-C to G-3CA. The reason for this increasing peak intensity, at the characteristic bonds of caffeic acid at around 1200 cm^{-1} and 1500 cm^{-1}, was the rise in caffeic acid concentration. Therefore, successful encapsulation of caffeic acid were done. Similarly, in a study of lentil flour electrospun nanofibers with gallic acid, the comparison of nanofibers with and without gallic acid showed a different peak intensity, which corresponded to the successful encapsulation (Aydogdu et al., 2019).

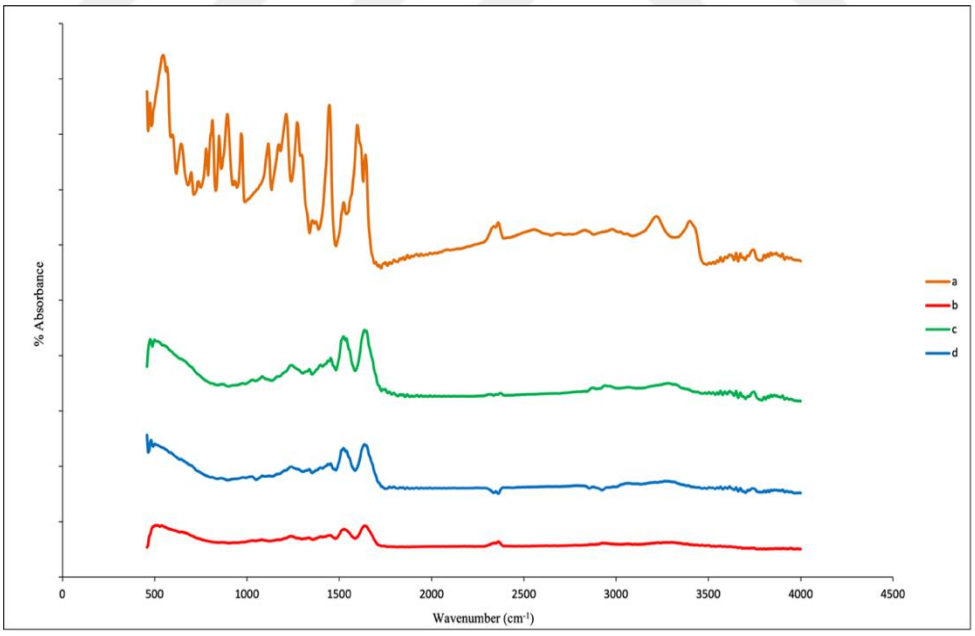


Figure 3.3 FTIR spectra of the samples. (a) pure caffeic acid, (b) 2% caffeic acid loaded film, (c) 3% caffeic acid loaded films, (d) control film.

3.1.2.5. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was done in order to examine the changes in weight loss according to the temperature. Figure. 3. 4(A) and (B) showed the curve of weight loss versus temperature and the rate of material weight changes upon heating for the films, respectively. The first weight loss was observed around 30-150˚C and can be evaluated as evaporation of residual moisture (Raeisi et al., 2021). Similarly, in a study about developing gelatin-based bactericidal wound dressing material by electrospinning, the first weight loss was between 40 and 175°C with a loss of 8.29% (İnal & Mülazımoğlu, 2019). For this study, weight losses at 150°C were 10.82%, 8.49% and 9.16% for G-C, G-2CA and G-3CA, respectively. It can be said that the first weight loss was not significantly affected from the addition of caffeic acid. In another study, which developed ZnO loaded gelatin-based fibers by electrospinning, weight loss for the first step was found as approximately 8.97% for 0%, 0.1% and 0.25% ZnO added samples (Y. Chen et al., 2019). The first weight loss step may be affected from different bond formations of water and protein. The first bond formation type, which water is bounded by high energy sorption centers, takes place in collagen triple helix, and has an important effect on stabilization of collagen helical structure by intramolecular bonds. The second one, which also helps to improve stabilization, can be defined as absorption of water by polar groups of gelatin and collagen macromolecules, and bound to proteins via hydrogen bonds, takes place out of the helical part, unlike the first one. Finally, the third one is the water absorbed by proteins for giving polymolecular layers. It was composed of the overall bound water content of the gelatin and collagen, as well as the structural water content of them (Correia et al., 2013). The second degradation step started at around 200°C, as can be seen more clearly in Figure 3. 4 (B). Then, it reached a peak at approximately 410°C, and continued to around 550°C. This weight loss step can be evaluated as the thermal decomposition of gelatin because of the breakage of protein chain and peptide bond (Khezri et al., 2021). Likewise, in a research, which studied curcumin added gelatin and chitosan based nanofibers by electrospinning, second degradation step was

between at around 250°C and 600°C (Duan et al., 2023). In addition, maximum degradation temperature of G-C, G-2CA and G-3CA were 414°C, 410°C and 412°C, respectively. At the end of this analysis, approximately 25% of the weight remained for all samples.

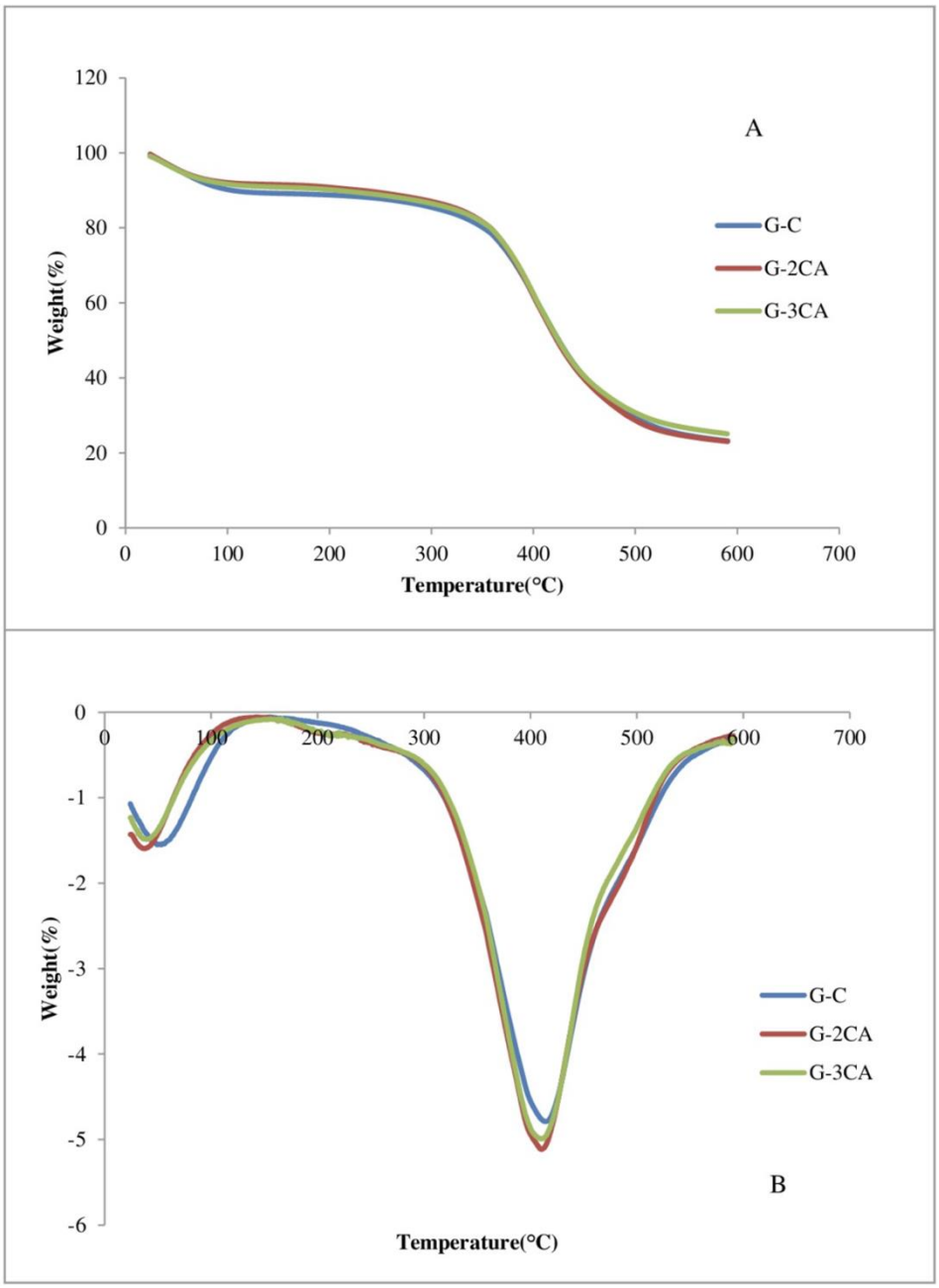


Figure 3.4 (A) Thermogravimetric curves and (B) DTG curves of samples.

3.1.2.6. Lipid Oxidation

Lipid oxidation causes both a change in color and the formation of rancidity, diminishing both consumer acceptance and the economic value of the food. Consequently, it becomes critical to extend the shelf life of oxidation-susceptible foods like meat, oils, and nuts by reducing lipid oxidation rates (Yildiz, Bayram, et al., 2021). The influence of centrifugally spun caffeic acid added nanofiber as an active film on susceptibility of olive oil to lipid oxidation was examined to assess the film's suitability for oil storage. The lipid oxidation outcomes for the control (oil without film) and oil containing caffeic acid-loaded gelatin films during a 14-day storage period in an air atmosphere at 40°C are depicted in Figure 3.5.

POV served as an indicator of oil oxidation, directly correlating with food quality, where higher POV in stored oil indicated increased oxidation, thus decreased quality (Yildiz, Bayram, et al., 2021). Hydrogen peroxides, which are the initial outcomes of lipid oxidation play a key role in primary oxidation processes. They are odorless and flavor-neutral products resulting from the reaction between oxygen and unsaturated fatty acids (Park & Kim, 2016). Even though hydrogen peroxides are intermediate compounds in the process of lipid oxidation, they tend to be quite stable. They serve as reliable indicators of lipid oxidation in food samples, as long as the sample hasn't reached an advanced stage of oxidation. The mechanism for detecting peroxides in oil sample is the generation of iodine by the reaction between peroxides and the iodide ion, in the presence of starch and acidic medium (Barriuso et al., 2013). According to the European Commission Regulation (1991) *No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis* the peroxide limit for refined olive was reported as 5 meq O_2/kg. Oils with elevated peroxide levels can lead to food toxicity and contribute to various diseases, including cancer, atherosclerosis, and Alzheimer's disease (Zhang et al., 2021). The initial POV of oil samples were 0.4423 ± 0.0274 meq O_2/kg oil (Figure 3.5 (A)). The data reveals a consistent increase in olive oil's POV over time. At the end of the 14-day accelerated shelf-life study, POV ranged between 1.30 ± 0.19 and 2.123 ± 0.51 meq O_2/kg oil for

G-3CA films and control films, respectively, indicating a significant prevention of peroxide formation with the addition of films. TPC and AA showed an increase with higher caffeic acid addition, potentially leading to a slower rise in POV, resulting in values falling below the critical limit. This phenomenon, indicating substantial radical scavenging capabilities and superior resistance to oil oxidation, aligns with prior research findings (Rangaraj et al., 2021; Wu et al., 2019).

Upon further oxidation of primary lipid oxidation products (hydrogen peroxides), secondary oxidation products are released, including aldehydes, ketones, epoxides, hydroxy compounds, oligomers, and polymers, characterized by diverse physicochemical properties such as volatility, polarity, and molecular weight (Barriuso et al., 2013). MDA, among the formed aldehydes, stands as one of the most prevalent and commonly utilized markers for oxidation. MDA amount is identified through the reaction between the monoleic form of MDA molecule and the active methylene groups of TBA at high temperature and low pH, distinguished by their peak absorbance at 532 nm. The analysis conducted at the end of the initial week revealed that the TBARS values of olive oils, whether with or without film, were statistically similar to each other and to the values measured on day 1 (Figure 3.5 (B)). After the two-week storage time, the control sample without film exhibited a greater increase in TBARS values compared to the samples with films. This effect cannot be solely explained by the addition of film to olive oil, as the TBARS value was not increased by films with 3% caffeic acid to the extent observed with 2%. The investigation of fish lipid model systems has explored how caffeic acid inhibits lipid oxidation, acting as an antioxidant, thus diminishing lipid oxidation (Yu et al., 2013).

Several aldehydes, not just malondialdehyde, arise during secondary lipid oxidation. The PAV method detects these aldehydes, especially α-unsaturated ones, 2-alkenals and 2,4-dienals. This mechanism occurs with the reactivity of the aldehyde carbonyl bond, that is on the amine group of *p*-anisidine, by creating a Schiff base that absorbs light at 350 nm (Barriuso et al., 2013). PAV shows a strong correlation with peroxide content and TBA, which proves its utility in estimating the overall oxidation in food. Thus, PAV is a measure utilized to assess the level of secondary oxidation products,

particularly aldehydes. They formed in oils due to decomposition during storage, aiding in evaluating the extent of degradation and rancidity in oils. Higher PAV indicates increased levels of oxidative breakdown products, especially when oils are exposed to high temperatures, light, or prolonged storage (Šimat et al., 2020). The initial PAV of oil samples was 10.694 ± 0.088. PAV consistently increased over the storage period for all samples (Figure 3.5 (C)). It signified the degradation of primary oxidation products in later stages of oxidation for all samples except those with high levels of added caffeic acid, specifically the G-3CA sample. The G-3CA sample maintained its initial PAV value, whereas the G-2CA sample showed a slight increase in 14 day accelerated storage. Caffeic acid appears to have lessened or postponed the generation of α-unsaturated aldehydes by interacting with hydroperoxides, thereby producing more stable compounds that are less susceptible to decomposition and subsequent reactions. Similar effects of gallic acid (Yildiz, Bayram, et al., 2021), mangosteen peel extracts (Chong et al., 2015), and orange peel extract (Rashid et al., 2022) have confirmed these results.

A good correlation was identified between POV and TBARS values, exhibiting a Pearson correlation coefficient of 0.963 ($p<0.05$).

The determination of the TOTOX value (Figure 3.5 (D)) serves as an empirical evaluation for the overall oxidation rate of the oils, with values below 30 indicating freshness and high quality in edible oils (El Idrissi et al., 2023). As shown in Figure 3.5 (D), the TOTOX values of the oils increased from 11.579 ± 0.142 to 27.980 ± 1.580, 17.887 ± 0.935, and 15.390 ± 0.804 after 14 days of storage for the control sample, G-2CA, and G-3CA, respectively. The TOTOX value of control olive oils approached the near-limit threshold of 30 in rapid shelf-life analysis, while oils coated with nanofibers containing 2% or 3% caffeic acid significantly remained well below this limit. This indicated the slow oxidation and superior oil quality. Yildiz, Bayram, et al. (2021) observed a reduction in overall olive oil oxidation by up to 20% through gallic acid-containing films, while this study exhibited a 45% reduction. The difference is attributed to variations not only in phenolic acids but also in the film fabrication method. Yildiz, Bayram, et al. (2021) utilized the casting method, whereas

this study employed centrifugal spin to produce the film and encapsulate caffeic acid within nanofibers. The porous structure and high surface area of these nanofibers might account for this discrepancy.

Overall, although both G-2CA and G-3CA lowered the lipid oxidation values in comparison with the control sample, the highest decrease was observed in G-3CA with 45% reduction of TOTOX values. This result was correlated with the highest antioxidant activity in G-3CA. On the other hand, although encapsulation efficiency of G-2CA was higher than G-3CA, total encapsulated caffeic acid content was higher in G-3CA. Therefore, the lowest lipid oxidation values for 3% caffeic acid concentration was expected.

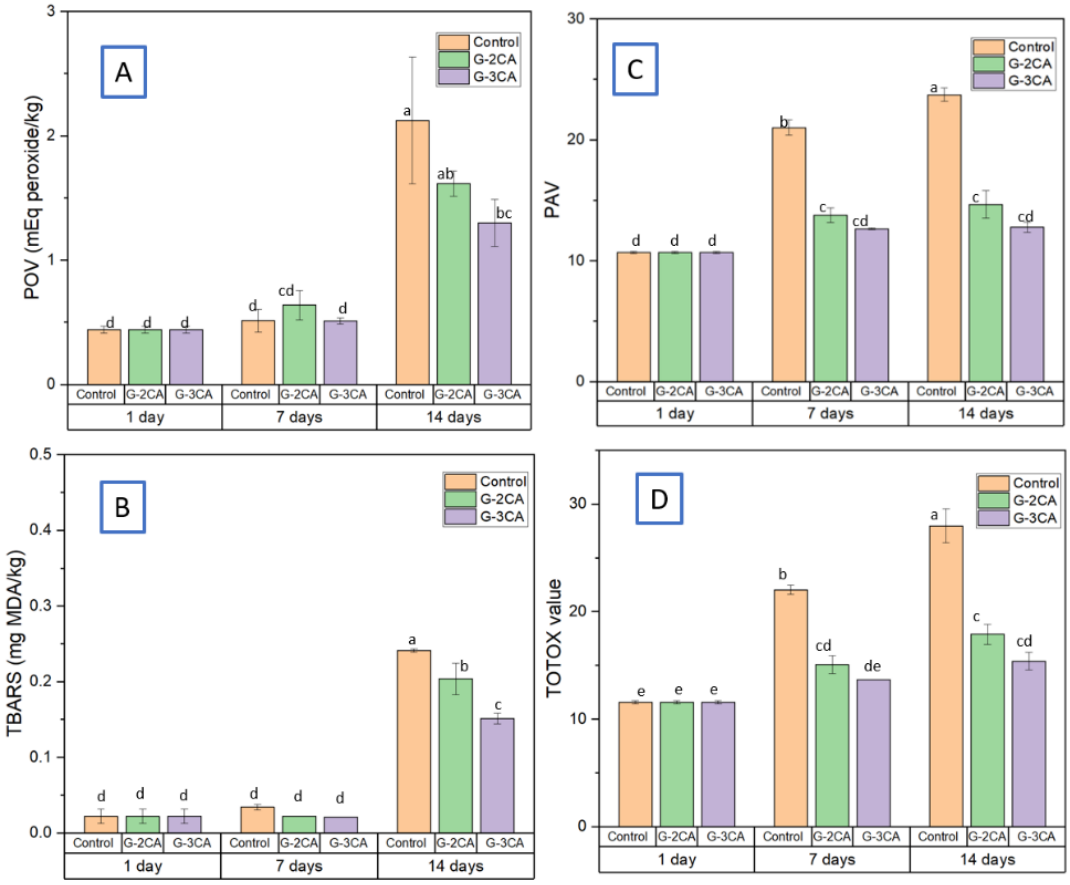


Figure 3.5 (A) POV, (B) TBARS values, (C) PAV, and (D) TOTOX values of oil samples without fibers (control) and with fibers (G-2CA and G-3CA) at 1st, 7th and 14th days.

3.1.2.7. Biodegradability of fibers

Biodegradability analysis was done in order to examine the degradation time of fibers in the soil and to verify the possibility of these fibers being an alternative to the synthetic food packages. The chemical changes and the decrement in mechanical properties are some indicators of the biodegradation. In addition, biodegradation is affected from some environmental factors such as temperature and moisture (da Silva Filipini et al., 2020). In order to examine the change in the area of films, visual observation was done every day. The decrease in the film area was considered as the degradation. According to Figure 3.6, which shows the pictures of film samples for five days, degradation can be seen prominently in the second day. In other words, there was more reduction in the area of films at the end of the second day, as compared to the first day. At the end of the third day, the change was not only the decrease in the area, but also in the porous and soil stickled film surfaces. Although similar structural changes occurred in all samples, if the decrease in film area was evaluated visually, degradation of control film (G-C) was more as compared to caffeic acid addition films (G-2CA and G-3CA). Similar to this result, in a research about gelatin and corn starch films with corn stigma extract, which has antioxidant activity, degradation rate of control film was found as higher when compared to active agent added films (Boeira et al., 2022). Finally, after four days, films were seen as a soil residue and the initial square shape could not be determined. As a result, films developed in this study, had a promising and important outcome in terms of being biodegradable and environmentally friendly.

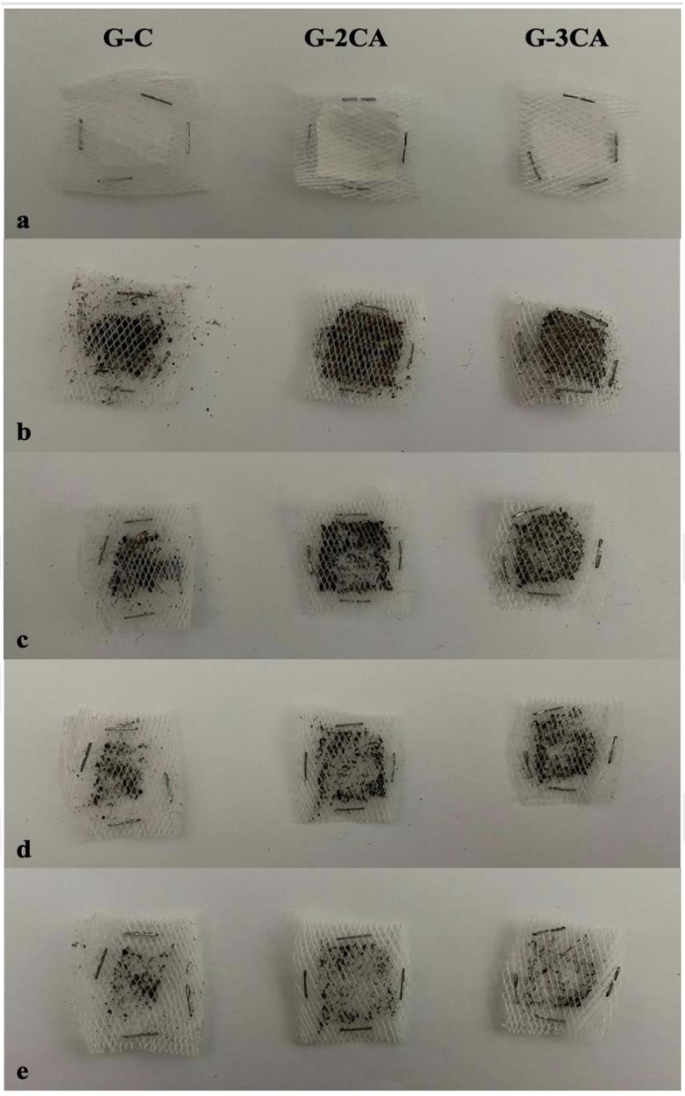


Figure 3.6 Biodegradability of films at the (a) beginning, (b) 1st day, (c) 2nd day, (d) 3rd day and (e) 4th day; order of the samples in each row: G-C, G-2CA and G-3CA.

3.2. Fabrication of Gelatin/Bay laurel leaf essential oil fibers by centrifugal spinning

Preliminary experiments showed that lower concentrations of gelatin resulted in low viscosity, as a result bead formation during spinning process. Moreover, addition of BLLEO affected the spinnability of solutions. Therefore, optimization study was conducted with the parameters of flow rate of the solution, gelatin and BLLEO concentration.

3.2.1. Optimization of fiber formulations

The experimental design to optimize the fiber samples by considering three factors according to the responses can be seen in Table 3.3. It was not possible to produce fibers at gelatin concentration of 25% with spun at a flow rate of 10 mL/h. This could be attributed to the high viscosity of the solution, which cannot be extruded at a slow flow rate, resulting in a lack of fiber formation. Consequently, the samples with a gelatin concentration of 25% at 10 mL/h were omitted from Table 3.3.

Fitted second order polynomial equations for the responses were represented in Table 3.4 with coefficient of determination (R^2), adjusted R^2, p-value and lack of fit data. After performing ANOVA, only the factors that had a significant impact on the responses were chosen, except for the main factors. The lower p-value, higher R^2, and adjusted R^2 values, along with the insignificant lack of fit, suggested that the reduced full second-order models adequately represented the response variables. The coefficient of determination of models were 0.8399, 0.9586 and 0.9198 for diameter, total phenolic content, and antioxidant activity, respectively.

Table 3.3 Experimental data and their response in regression design

Run	*Factor 1* X_1: *Flow rate (mL/h)*	*Factor 2* X_2: *Gelatin concentration (%)*	*Factor 3* X_3: *BLLEO concentration (%)*	*Response 1* *Diameter (nm)*	*Response 2* *Phenolic content (mg GAE/g)*	*Response 3* *AOA(%)*
1	15	20	1	2791.9±12	1.740±0.001	15.550±0.495
2	15	20	3	3109.1±11	2.102±0.001	17.975±0.001
3	15	20	5	2423.0±87	2.570±0.014	22.700±0.141
4	10	20	1	2280.1±63	1.235±0.007	12.750±0.495
5	10	20	3	2209.0±16	1.600±0.085	11.100±1.131
6	10	20	5	2259.3±12	1.930±0.099	12.200±0.141
7	20	20	1	2259.9±21	1.624±0.001	12.979±0.001
8	20	20	3	2315.0±36	1.985±0.078	18.100±0.001
9	20	20	5	1956.4±11	2.245±0.078	24.250±1.770
10	15	25	1	2947.7±61	0.985±0.007	13.500±0.001
11	15	25	3	2815.0±23	1.490±0.127	16.650±1.344
12	15	25	5	2580.0±37	1.865±0.050	20.800±2.970
13	20	25	1	3571.8±56	1.100±0.000	13.250±0.354
14	20	25	3	3499.0±36	1.410±0.099	19.800±0.283
15	20	25	5	3778.0±20	1.695±0.064	25.950±1.770

Table 3.4 Fitted second order polynomial equations for the responses

Response	*2nd order polynomial equations (Quadratic model)*	R^2	*p-value*	*Adjusted* R^2	*Lack of fit*
Diameter (nm)	$14591-506X_1$***[b]$-805X_2$****[a]$-43.1X_3-19.8X_1X_1$***$+54.63X_1X_2$****	0.8399	0.000	0.7699	0.178
TPC (mg GAE/g)	$0.611+0.4175X_1$****$-0.1241X_2$****$+0.181X_3$****$-0.01259X_1X_1$****	0.9586	0.000	0.9447	0.05
AOA (%)	$-5.22+2.674X_1$***$-3.021X_3$***$-0.0959X_1X_1$***$+0.305X_1X_3$****	0.9198	0.000	0.9019	0.281

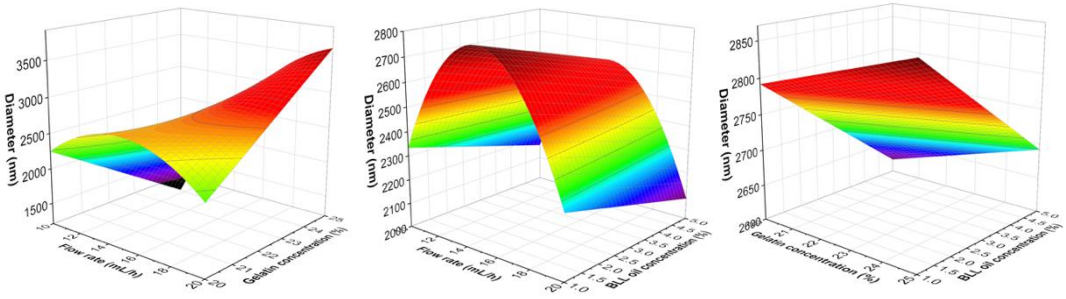
X_1: Flow rate (mL/h), X_2: Gelatin concentration (%), X_3: BLLEO concentration (%)

[a] Significant at %0.01 level.

[b] Significant at %0.1 level.

Figure 3.7 illustrates the 3D surface plots showing the effects of independent variables, flow rate, gelatin and BLLEO concentration on fiber diameter. It can be observed that increasing the gelatin concentration resulted in thicker fibers (Figure 3.7 (a)). Zhang and Lu (2014) figured out that larger fiber diameters are formed with the high Berry number, which is a dimensionless term used in electrospinning and related to the solution concentration.

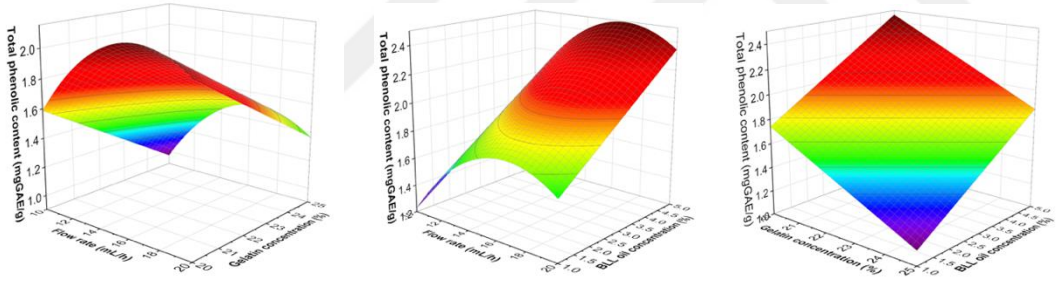
Moreover, the increase in the concentration of solution might enhance the cohesion and macromolecular chain entanglement, therefore thicker fibers were obtained (Pakolpakçıl et al. 2023). Increasing the flow rate led to thickened fibers when BLLEO concentration was 3% (Figure 3.7 (a)). However according to the Figure 3.7 (b), at the concentration of 5% BLLEO, thinner fibers were obtained at higher flow rates, when the gelatin concentration was 20%. This difference might be due to the difficulty of pushing the solution inside the syringe with higher BLLEO concentration when the flow rate was low. In Figure 3.7 (c), lower gelatin concentration with enhancing BLLEO content ended up with thin fibers. Fibers with thinner diameters were more desirable since encapsulating properties and accessible surface areas could be improved by reducing the fiber diameter (Pakolpakçıl et al. 2023). In this aspect, selecting low gelatin and high BLLEO concentration at faster flow rate resulted in minimized fiber diameter, which can be considered as the optimum condition. According to Figure 3.7 (b), at the concentration of 5% BLLEO, minimum fiber diameter was obtained at higher flow rates, when the gelatin concentration was 20%.



(a) (b) (c)

Figure 3.7 Effect of different parameters on the average fiber diameter. (a) Flow rate (mL/h) and gelatin concentration (%) when BLLEO concentration 3%, (b) Flow rate (mL/h) and BLLEO concentration (%) when gelatin concentration 20%, (c) Gelatin concentration (%) and BLLEO concentration (%) when flow rate 15 mL/h.

The effect of flow rate, gelatin and BLLEO concentration on TPC was given in Figure 3.8. TPC arises because of the BLLEO content, therefore enhancement of TPC by increasing BLLEO concentration was expected. Figure 3.8 (a) shows that when gelatin concentration was 20% and flow rate was 15 mL/h with 5% BLLEO concentration, TPC was maximum. However, TPC was adversely affected from lowering the flow rates and the optimum TPC concentration was found around a flow rate of 18 mL/h when gelatin concentration was 20% (Figure 3.8 (b)). At low flow rates, the process for reaching the solution to the spinneret takes more time. In this time interval, BLLEO might be reduced by volatilization which results in lower TPC. The increase in gelatin concentration decreased TPC, indicating the release of phenolic compounds was more in 20% gelatin concentration. This might be caused by the protein and phenolic compound interaction that can inhibit the active sites and as a result TPC was reduced (Aliabbasi et al. 2021). The maximum TPC values were obtained as 2.4 mg GAE/g with 20 mL/h flow rate, 20% gelatin and 5% BLLEO.



(a) (b) (c)

Figure 3.8 Effect of different parameters on the total phenolic content (TPC). (a) Flow rate (mL/h) and gelatin concentration (%) when BLLEO concentration 3%, (b) Flow rate (mL/h) and BLLEO concentration (%) when gelatin concentration 20%, (c) Gelatin concentration (%) and BLLEO concentration (%) when flow rate 15 mL/h.

In line with TPC results, AOA showed a similar behavior as demonstrated in Figure 3.9. The highest AOA was obtained with higher BLLEO concentration and faster flow rate. As shown in Table 3.4, the gelatin concentration did not significantly affect the

AOA of the fibers. In other words, the essential oil: gelatin ratio was lowered in the fiber formed by high concentration of gelatin, leading to low TPC.

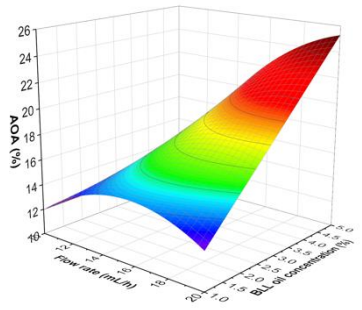


Figure 3.9 Effect of different parameters on the antioxidant activity (AOA). Flow rate (mL/h) and BLLEO concentration (%) when gelatin concentration 20%.

Table 3.5 presents the predicted and optimized values along with their corresponding p-values. For the optimization, all independent variables were set within their ranges, aiming to minimize fiber diameter and maximize TPC and AOA to determine the predicted values. The model values for diameter were 2093 ± 125.0 (nm), TPC was 2.349 ± 0.043 mg GAE/g, while AOA was 25.301 ± 0.639%. The experimental values closely matched the predicted values for all responses (Table 3.5) as evidenced by 2 sample t test p values exceeding 0.05. Desirability score was 0.89, confirming the model's reliability.

Table 3.5 Predicted and experimental values using optimized conditions for minimum diameter, and maximum TPC and AOA.

Response	*Predicted value*	*Experimental value*	*p-value*
Diameter (nm)	2093 ± 125.0	1956.4 ± 11.2	0.201
Total Phenolic Content (mg GAE/g)	2.349 ± 0.043	2.245 ± 0.078	0.335
Antioxidant activity (%)	25.301 ± 0.639	24.250 ±1.770	0.568

Columns with different letters are significantly different ($p \leq 0.05$).

3.2.2. Rheological properties of solutions

In Figure 3.10, shear stress versus shear rate curves of fiber forming solutions were given for control, BLLEO-1, BLLEO-3 and BLLEO-5. For all samples, the flow

curves exhibit a linear relation between shear stress and shear rate, explaining the Newtonian behaviour. The shear rate range in which gelatin exhibits Newtonian behavior was specified as 10 to 350 s^{-1}, which showed correlation with previous work done by using mammalian and fish gelatin (Leuenberger 1991). For this study, Newtonian behaviour was observed at the range of 0.1 to 100 s^{-1}. Similarly, Newtonian behaviour was observed for fiber-forming solutions of electrospun gelatin fibers incorporated with saffron extract, which uses Type A bovine gelatin in a concentration of 25% (w/w) prepared with formic acid (Golpira et al., 2021). Table 3.6 shows the viscosity values of solutions, ranging between 0.376± 0.01 and 0.433± 0.01 Pa.s, with no significant difference. Similar result was obtained in the study of gelatin-based films with ginger essential oil (Alexandre et al., 2016).

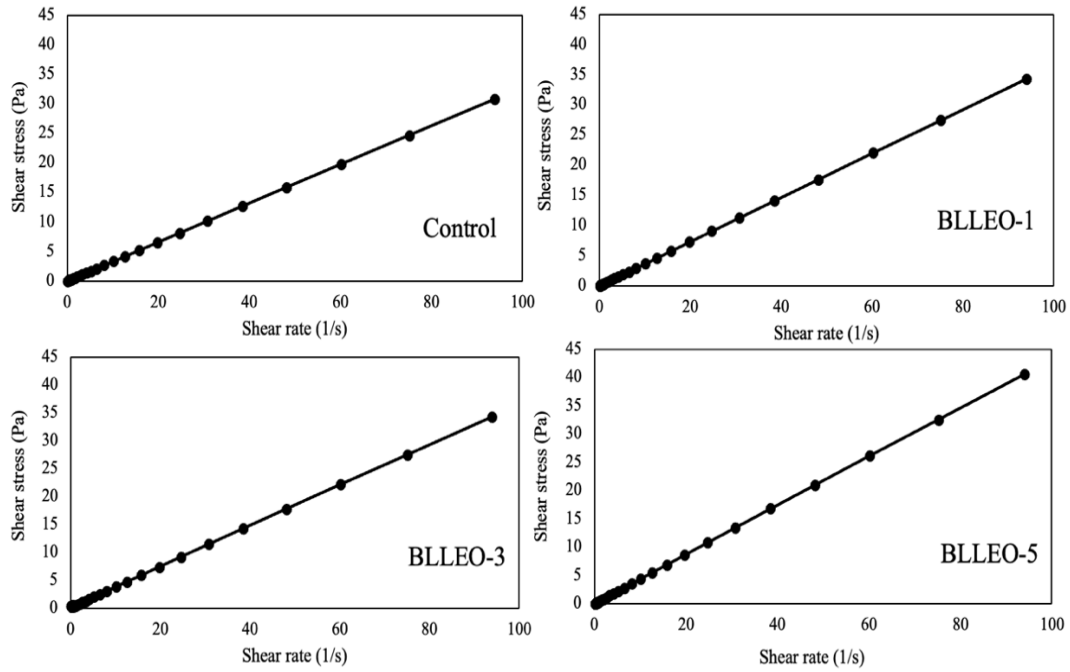


Figure 3.10 Shear stress versus shear rate curves of fiber sample solutions

Table 3.6 Viscosity, Average diameter, Water Vapor Permeability (WVP), Melting Temperature (T_m) and Melting Enthalpy (ΔH_m) of fibers

Samples	*Viscosity (Pa.s)*	*Average Diameter (nm)*	*WVP* $\times 10^{-12}$ *($g.m^{-1}.s^{-1}.Pa^{-1}$)*	*T_m (°C)*	*ΔH_m (j/g)*
Control	0.423 ± 0.01^a	1541.4 ± 103.9^a	4.265 ± 0.119^b	198.67	143.18
BLLEO-1	0.376 ± 0.01^a	2259.9 ± 21.0^a	6.019 ± 0.539^a	111.29	74.37
BLLEO-3	0.424 ± 0.00^a	2315.0 ± 361.0^a	6.033 ± 0.415^a	140.54	95.54
BLLEO-5	0.433 ± 0.01^a	1956.4 ± 11.2^a	6.210 ± 0.472^a	151.03	131.74

BLLEO-1, BLLEO-3, and BLLEO-5 represent fibers containing 1%, 3%, and 5% BLLEO.

Columns with different letters are significantly different ($p \leq 0.05$).

3.2.3. Characterization of fibers

3.2.3.1. Fiber morphology

Figure 3.11 shows the fiber diameter distribution graphs and FESEM images, with a homogenous and bead free structure. Addition of essential oil did not affect the smooth structure of fibers, as found in the study of encapsulation of cumin essential oil in electrospun zein fibers (Ghasemi et al., 2022). Average diameter values were given in Table 3.6, ranging from 1541.4 ± 103.9 to 2315.0 ± 361.0 nm. Although there was no significant difference observed in diameter values, fibers of control sample had the thinnest diameters. Addition of BLLEO displayed an increment in fiber diameter in comparison to the control. Similar values were obtained in a study of lemon peel essential oil added centrifugally spun gelatin fibers, with an average fiber diameter of

pure gelatin and incorporated with 1%, 3.5%, 7% lemon peel essential oil, 1717.84 ± 38.21, 2337.12 ± 62.79, 2365.52 ± 7.65 and 2394.55 ± 44.25 nm, respectively (Doğan et al., 2022b). Also, in other studies about encapsulating essential oils, it was stated that addition of essential oil showed an increment of average fiber diameters (Ansarifar & Moradinezhad, 2022; Doğan et al., 2022a). Diameter sizes of fibers were directly correlated with the viscosity values (Aslaner et al., 2021). In the scope of this

information, both viscosity and diameter values were found to be not significantly different.

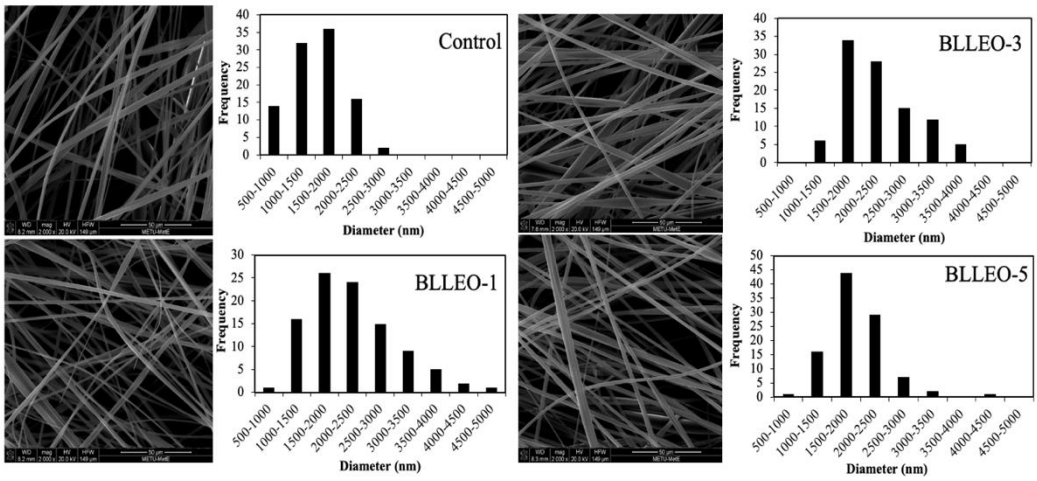


Figure 3.11 FESEM images and fiber size distributions of fibers

3.2.3.2. Total Phenolic Content (TPC), Antioxidant Activity (AOA) and Encapsulation Efficiency (EE)

Antioxidant activity (AOA) and total phenolic content (TPC) of fibers were shown in Table 3.7. For control sample, which does not contain BLLEO, AOA and TPC values were not detected since there was no active agent to form an antioxidant or phenolic property. In the previous studies about addition of various essential oils to the gelatin film-forming solutions (Alparslan, 2018), and development of lemon essential oil added gelatin/chitosan film (Tügen et al., 2020), it was also mentioned that control sample without any essential oil showed an undetectable AOA and TPC content. On the other hand, there was a rising trend for both AOA and TPC values, proportionally

with the increase in BLLEO essential oil concentration from 1% to 5%. Similar trend was observed in the study of electrospun gelatin fibers with peppermint and chamomile essential oil (Tang et al., 2019). AOA values of fibers were found as 12.98 ± 0.01%, 18.10 ± 0.01% and 24.24 ± 1.77% for BLLEO-1, BLLEO-3 and BLLEO-5, respectively. According to the study conducted by Tural et al. (2020), antioxidant activity of 1% laurel essential oil added protein-based film was given as 10.71 ±

0.24%. This finding was slightly lower than AOA of BLLEO-1 sample, 12.98 ± 0.01%, by having the same concentrations of BLLEO, indicating the effective encapsulation of BLLEO by centrifugal spinning.

Similar trend as in AOA values was observed in TPC. The highest TPC value was found in BLLEO-5 sample, then continued with BLLEO-3 and BLLEO-1, as 2.25 ± 0.08, 1.99 ± 0.08, and 1.62 ± 0.01 mg GAE/g, respectively. Similar trend was observed in other researches such as TPC of gelatin films containing 2%, 4%, 6% and 8% *Origanum onites L.* essential oil (Kilinc et al., 2021), and gelatin-sodium alginate based films with concentration of 1%, 3%, 5% yarrow essential oil (Karami et al., 2022). Moreover, in a research about developing an edible film by using maize starch and rice protein incorporated with 1% LLEO, TPC value was given as 0.26846 mg GAE/g film sample (Kurtfaki and Yildirim-Yalcin 2023). As compared to this study, TPC of maize starch and rice protein film was found lower, even though the same concentration of LLEO was added. This difference can be explained by two possible reasons. One of them was the encapsulation method of LLEO, which was pouring and drying film in trays for lower TPC, and centrifugal spinning for higher TPC. Another was the applying heat treatment to films, resulted in lower TPC as compared to this study, which conducted without heat treatment.

Encapsulation efficiency (EE) results can also be seen in Table 3.7, with the values of 94.77 ± 1.44, 80.96 ± 0.02, and 54.70 ± 0.35% for BLLEO-1, BLLEO-3 and BLLEO-5, respectively. Results showed an opposite trend not only with BLLEO concentration, but also with the AOA and TPC. Increment of the BLLEO concentration resulted in reducing EE, as mentioned in other studies (Göksen et al., 2020; Hadad & Goli, 2019). This result was expected because of the limited capacity of the polymer matrix to entrap the active agent (Gómez-Mascaraque et al., 2017). In addition, Fonseca et al. (2020) explained that addition of less essential oil can come up with more efficient encapsulation. When the comparison was done between BLLEO-1, BLLEO-3 and BLLEO-5, significant difference can be observed in the EE values. The reason of the lowest EE belonged to BLLEO-5 might be attributed to the higher volatilization of BLLEO with more concentration since the capacity of maximum entrapped essential

oil might be exceeded. Besides, this loss can also be occurred due to the lower formation and accumulation of fiber during the centrifugal spinning process by increasing the concentration of active agent (Vera et al., 2018). Still, by having EE value of 94.77 ± 1.44% for 1% essential oil added sample, an efficient encapsulation was done in comparison to the other study with the same concentration of cinnamaldehyde, limonene, and eugenol essential oil (Mahmood et al., 2023).

Table 3.7 Antioxidant Activity (AOA), Total Phenolic Content (TPC), Encapsulation Efficiency (EE) and Antimicrobial Inhibition Zone of Fibers

Samples	*AOA (%)*	*TPC (mg GAE/g)*	*EE (%)*	*Inhibition Zone (%)*	
				E. coli	*S. aureus*
Control	N.D.	N.D.	N.D.	N.D.	N.D.
BLLEO-1	12.98 ± 0.01^{b}	1.62 ± 0.01^{b}	94.77 ± 1.44^{a}	1.64 ± 0.12^{b}	2.01 ± 0.53^{b}
BLLEO-3	18.10 ± 0.01^{ab}	1.99 ± 0.08^{a}	80.96 ± 0.02^{b}	2.01 ± 0.03^{b}	7.01 ± 0.19^{a}
BLLEO-5	24.24 ± 1.77^{a}	2.25 ± 0.08^{a}	54.70 ± 0.35^{c}	2.89 ± 032^{a}	7.87 ± 1.47^{a}

BLLEO-1, BLLEO-3, and BLLEO-5 represent fibers containing 1%, 3%, and 5% BLLEO.

Columns with different letters are significantly different ($p \leq 0.05$).

N.D. means “Not Detected”.

3.2.3.3. Water Vapor Permeability (WVP)

WVP values of fibers were given in Table 3.6, ranging from 4.265 ± 0.119 to 6.210 ± 0.472 $\times 10^{-12}$ g $m^{-1}s^{-1}Pa^{-1}$, showing a significant difference between control fiber and essential oil added ones. There was no significant change between the WVP values of fibers with essential oil at different concentrations. The lack of significant impact on WVP with the increase in BLLEO concentration is a favorable outcome. This is because, even as the BLLEO concentration increases to reduce lipid oxidation or enhance antioxidant activity, it will not adversely affect WVP. Similar finding was observed in the study of anchovy by-product protein-based films with laurel essential

oil. Adding 0.5%, 1% and 1.5% concentration of laurel essential oil had no significant difference among them; however, compared with the control sample, significant increase was seen (Tural et al., 2020). This increasing behavior was not preferred for packaging industry since excess moisture transmission may not be prevented and therefore the shelf life and the quality of the food product may be affected adversely (Kyeong Yoon et al., 2023). The reason for increasing trend in WVP values with the addition of essential oil was the formation of a porous and heterogenous surface due to an interaction between the essential oil and the hydrophobic part of gelatin (Nunes et al. 2021). Nevertheless, WVP values obtained in this study were still lower than the ones with cinnamon essential oil (Wu et al. 2017) and ginger essential oil (X. Li et al., 2022) added fish gelatin films, with the values of $1.57 \pm 0.04\ 10^{-11}$ g m^{-1} s^{-1} Pa^{-10} and $6.92 \pm 0.17\ 9\ 10^{-11}$ g m^{-1} s^{-1} Pa^{-1} for 1% essential oil concentration.

3.2.3.4. Fourier Transform Infrared Analysis (FTIR)

Figure 3.12 shows the FTIR spectrum of fiber samples with and without BLLEO. At the first sight, some intense peaks were observed at the wavelengths of 1247, 1516, 1636, 2878 and 2975 cm^{-1}, for all samples, regardless of the essential oil content. Wavelength of 1247,1516 and 1636 cm^{-1} corresponded to the Amide III, Amide II and Amide I bond, respectively, which can be attributed to the gelatin structure. Amide I bond demonstrates the stretching vibrations of C=O group, Amide II illustrates the C-N stretching and the N-H bending vibration, and Amide III occurs due to the N-H bending. The general interval for Amide I, Amide II and Amide III bond were known as 1600-1700, 1500-1600, and 1200-1400 cm^{-1}, respectively (Sizeland et al. 2018). Similar to this study, wavelengths of Amide I, Amide II and Amide III bonds of gelatin-based edible films incorporated with spearmint essential oil were found as 1640, 1549, and 1239 cm^{-1}, respectively (Kyeong Yoon et al., 2023). Mentioned two other intense peaks, 2878 and 2975 cm^{-1}, observed in most lipids and corresponded to the methylene asymmetrical and symmetrical C-H stretching vibration, respectively (Wu et al. 2017). As can be seen in Figure 3.12, these two peaks became more intense with the increasing BLLEO concentration. Similar wavelengths at 2856 and 2929 cm^{-1}, and

enhanced peaks as the increasing essential oil content were observed in the study of gelatin films with ginger essential oil (X. Li et al., 2022). For the characteristic vibrational band of BLLEO, two peaks can be observed at the wavelengths of 1053 and 1373 cm^{-1}, related to the cineole and 1,8-cineole, which were the most common compound in BLLEO. Wavelength of 1053 cm^{-1} indicates the stretching vibrations of C-O, whereas 1373 cm^{-1} related to the methyl CH symmetrical bending and stretching vibration of -C-O- (Göksen et al., 2020). In addition, a peak observed at 1733 cm^{-1}, except for control, may arise due to the C=O stretching vibration of ester or aldehyde carbonyl groups, which are the main existing chemical compounds in essential oils (Tural et al., 2020). Similarly, a peak was observed by Tongnuanchan et al. (2014), at 1733-1743 cm^{-1}, in fish gelatin films with basil and citronella essential oil. Moreover, it can be obviously seen that the intensity of the fluctuating peaks between 810 and 1344 cm^{-1}, owing to the essential oil, enhanced as the BLLEO concentration increased, as in line with the findings in the study of nanofiber development with essential oils by electrospinning (Göksen et al., 2020).

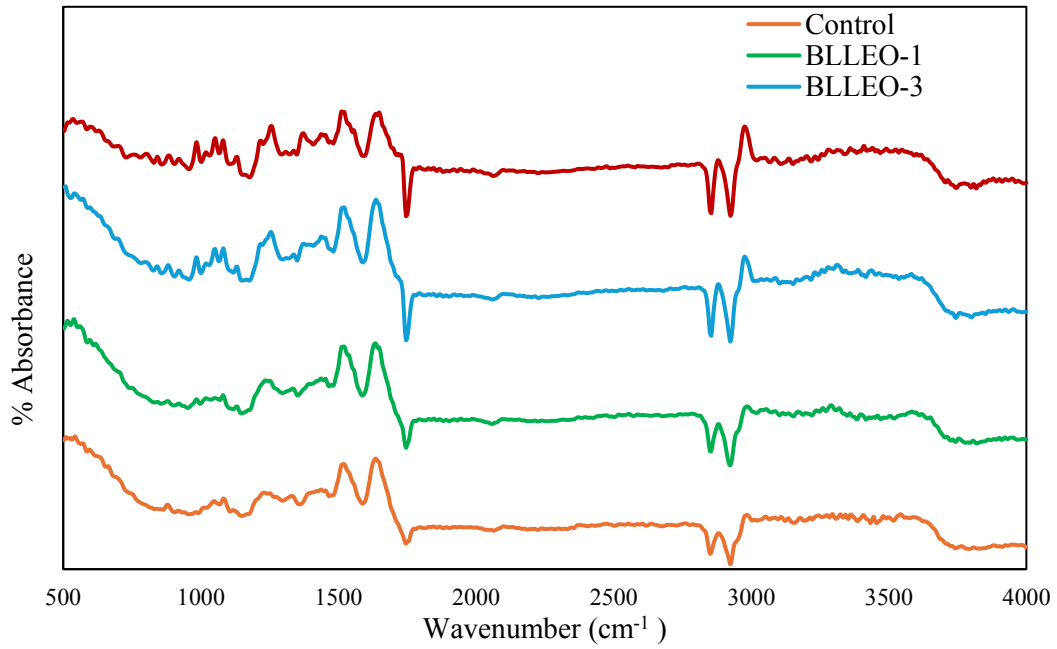


Figure 3.12 FTIR spectra of the fibers

3.2.3.5. Thermal Analysis (TGA and DSC)

Thermogravimetric analysis was given as weight loss versus temperature curves of fibers, in Figure 3.13. The first weight loss was observed between the range of 25°C and 100°C, attributed to the evaporation of adsorbed water molecules and other volatile compounds (Hosseini et al., 2016). At 100°C, weight loss of control, BLLEO-1, BLLEO-3 and BLLEO-5 was found as 5.045%, 5.925%, 5.561%, and 5.039%, respectively. According to these values and curves, there was not a significant change in the first step weight loss of samples. Similar weight loss was given in the range of 3.91% and 7.81% in the study of fish skin gelatin film with citrus essential oils (Tongnuanchan et al., 2012). The second weight loss, mainly corresponded to the protein decomposition or degradation (Nunes et al., 2021), started to occur from 177.69°C for control and BLLEO-1, whereas BLLEO-3 and BLLEO-5 occurred from 243.32°C. It can be said that increasing the BLLEO concentration led to an increase in the degradation temperature, however it reduced the weight loss. Enhancement of thermal stability of fibers with BLLEO can be explained by the interaction of essential oil and gelatin, due to the formation of stronger network (Ahmad et al., 2012). Similar findings were figured out in the study of centrifugally spun gelatin fiber incorporated with lemon peel essential oil (Doğan et al., 2022b). At the final, fibers had a remaining weight between 20.168% and 21.839%.

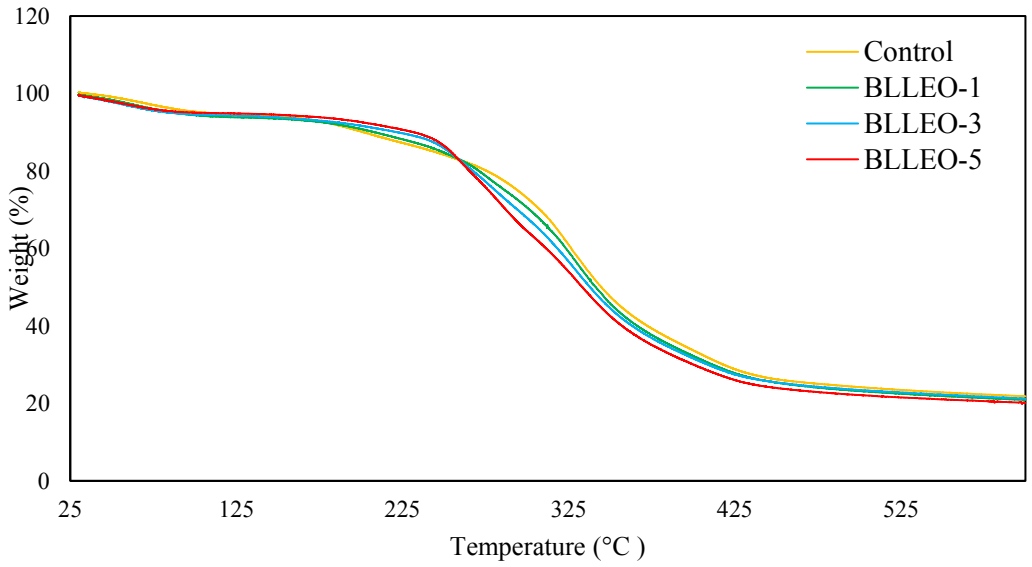


Figure 3.13 Thermogravimetric curves of fibers

DSC results of fibers with and without BLLEO were given as melting temperature (T_m) and enthalpies (ΔH_m) in Table 3.6. Melting temperature, which was the maximum temperature point, represents the destruction of ordered structure steadied by protein interactions (Tongnuanchan et al., 2014). Melting temperatures and enthalpies were ranged from 111.29 to 198.67°C, and from 74.368 to 143.18 J/g, respectively. Both showed a reduction with the presence of BLLEO, as compared to control fiber. This result can be caused by the enhanced free volume of the polymer network due to the plasticizing effect of essential oil (Hosseini et al., 2015). Oil, by being hydrophobic, might create a disruption of protein's molecular interactions and result in more mobile chain of gelatin structure (Tongnuanchan et al., 2016). Similarly, melting temperatures of gelatin films with *Origanum onites* L. essential oil were decreased from 222.7°C, which was the melting temperature of control, to around 200°C (Kilinc et al., 2021). In another study, enthalpy of hagfish skin gelatin-based film diminished from 195 to 181 J/g with the addition of 1% cinnamon bark essential oil (Kim et al., 2018). Low enthalpy values might be an indicator of weak film network since the ordered macromolecular structure was destroyed by the oil droplets by lowering the gelatin chain interactions (Tongnuanchan et al., 2016).

3.2.3.6. Lipid Oxidation

Lipid oxidation can be an important problem in most of the foods by triggering not only the quality and nutrition loss, but also the color, texture, flavor, and odor changes. Therefore, reduction of lipid oxidation is crucial to enhance the shelf life of food (Tian et al., 2013). The peroxide value (POV) is an indicator of lipid oxidation and determines the main primary lipid oxidation products, known as hydroperoxides (Chaijan, 2011). Higher POV may cause some human diseases such as cancer (Wang, Zhang, et al., 2019), therefore it is crucial to improve prevention methods from higher POV. In this aspect, BLLEO incorporated fibers were exposed to an oxidation process to analyze the possibility of being active packaging and demonstrated in Fig. 8 with 14-days storage period.

According to the Figure 3.14 (A), the highest POV, with 1.282 ± 0.009 meq O_2/kg oil, was detected in the control sample on 14th day. Conversely, the lowest POVs were obtained on 14th day, with the samples of BLLEO-3 and BLLEO-5, calculated as 0.241 ± 0.024 and 0.223 ± 0.008 meq O_2/kg oil with no significant difference, respectively. This result indicated the effect of essential oil on reducing lipid oxidation. In addition, reduction of POV with the increasing BLLEO concentration might be related to the AOA and TPC values. The relation between AOA, TPC and lipid oxidation can be explained by the migration of BLLEO phenolic content, which behave like hydrogen donors and inhibit the unsaturated fatty acid oxidation (Wang, Zhang, et al., 2019). Similar findings were mentioned in the study of Wu et al. (2019). Notably, in comparison to 7th day, the effect of BLLEO became more significant on 14th day, as it can be seen especially in BLLEO-3 and BLLEO-5.

During the storage period, primary oxidation products can be transformed to the secondary oxidation products such as aldehydes, epoxides, and ketones, by further oxidation (Abedi et al., 2015). Aldehydes, especially non-volatile ones, which are 2-alkenals and 2,4-dienals, can be detected by p-anisidine value (PAV) by the reaction between amine group of p-anisidine and carbonyl bond of aldehyde (Barriuso et al., 2013). PAV results were given in Figure 3.14 (B), with an obvious rise in control and BLLEO-1 sample. In comparison to the 1st day, control sample continued to increase its PAV, however BLLEO addition postponed the formation secondary oxidation products. BLLEO-1 showed a different trend on 7th and 14th day. Although there was a significant difference between PAV of control and BLLEO-1 on 7th day, no significant difference was observed on 14th day. On the other hand, BLLEO-3 and BLLEO-5 displayed a significant reduction both on 7th and 14th day. On 14th day, PAV result of BLLEO-3 and BLLEO-5 were 30%, 37% lower than control oil, while BLLEO-1 was almost the same. This finding may indicate that lower concentrations of BLLEO cannot be sufficient for the further storage times.

Another common secondary oxidation product, malondialdehyde (MDA), can be measured by using TBARS value (Baghdadi et al., 2019). According to the Figure 3.14 (C), increase in the TBARS values enhanced on 14th day as compared to 1st and 7th days. Enhancing BLLEO concentration did not show a difference at the end of the 7th

day, however on 14th day, significant reduction was observed in BLLEO-5, in comparison to control, indicating that formation of secondary oxidation products was diminished. As in line with this study, whey protein films with blended essential oils showed a reduction in TBARS values (Ribeiro-Santos et al., 2017). While there was no change on 7th day TBARS values of control, BLLEO-1 and BLLO-3 samples compared to the 1st day, a change was seen on 14th day. But for the BLLO-5 sample, a significant change was present on both the 7th and 14th days. It can be said that incorporating more BLLEO can fit the purpose of reducing lipid oxidation.

TOTOX values are considered as the overall lipid oxidation measurement including not only primary oxidation products, but also secondary oxidation products, since the calculation of it based on POV and PAV. TOTOX values had a rising trend as can be seen in Figure 3.14 (D), as well as the other lipid oxidation results. The effect of BLLEO was highlighted obviously on 14th day, with a wide difference between control and fibers with BLLEO, especially for 3% and 5% concentration. TOTOX values of

control, BLLEO-1, BLLEO-3 and BLEEO-5 were found as 34.473 ± 2.208, 33.578 ± 1.436, 22.758 ± 3.193, and 21.478 ± 0.749, respectively. Results were correlated with PAV since BBLEO-1 was not significantly difference from control sample, probably due to the volatilization of some essential oil amount. 1% BLLEO concentration became even less, therefore its effect on lipid oxidation cannot be seen significantly. At the 14th day, in comparison to control sample, the reduction in TOTOX values were 3%, 34% and 38% for BLLEO-1, BLLEO-3 and BLLEO-5, respectively.

In conclusion, although adding BLLEO reduced the lipid oxidation values, BLLEO-5 showed a significant result by lowering all the oxidation values mostly. This finding proved the enhanced AOA and TPC values with the highest concentration of BLLEO addition.

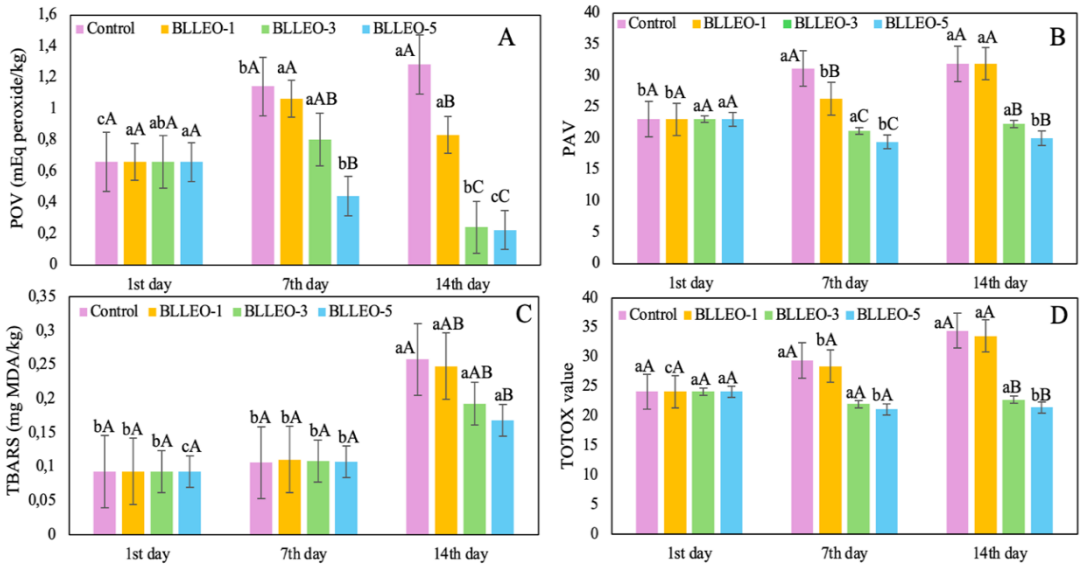


Figure 3.14 A) POV, (B) PAV, (C) TBARS, and (D) TOTOX values of oil samples without fibers (control) and with fibers (BLLEO-1, BLLEO-3, and BLLEO-5) at 1st, 7th and 14th days. The different lowercase letters indicate significant difference ($p<0.05$) in the means within the same BLLEO concentration on different days; different uppercase letters indicate significant difference ($p<0.05$) in the means within the same days.

3.2.3.7. Antimicrobial Activity

Antimicrobial activity of fibers was tested on *E.coli* and *S. aureus*, which are Gram-negative and Gram-positive, respectively. Control fiber did not reduce the microbial growth since there was no essential oil in it. Inhibition zones were given as % values, as can be seen in Table 3.7, with a different trend for *E. coli* and *S. aureus*. Although there was no significant difference between BLLEO-1 and BLLEO-3, BLLEO-5 showed a significant higher reduction of microbial growth area of *E. coli*, as compared to the fibers with 1% and 3% concentration of BLLEO, meaning that less than 5% concentration of BLLEO did not make a significant change in the inhibition area of bacteria. On the other hand, for *S. aureus*, BLLEO-3 and BLLEO-5 formed a significant inhibition area as compared to the BLLEO-1. In other words, increasing the BLLEO concentration from 1% to 3%, made a significant effect on gram positive

microbial growth, with a change in the inhibition zone from 2.014 ± 0.53% to 7.009 ± 0.19%, respectively. As a result, it is worth noting to say that BLLEO was more effective on *S. aureus* than *E. coli*. This result was also mentioned in the study of plant essential oil added cellulose fibers (Zhou et al., 2022), and similar finding was observed in study of gelatin based electrospun films with *Oliveria decumbens Vent* essential oil (Abbasi et al., 2021). It is known that Gram-positive bacteria are less resistant than Gram-negative bacteria, as in line with the results of this study. The main reason of this situation might be the lipopolysaccharide compounds that are placed in the Gram-negative bacteria's outer membrane. Through the bacteria cell wall, entrance of phenolic compounds present in the essential oil was expected because of their hydrophobicity. However, this entrance may be inhibited due to the mentioned lipopolysaccharide compounds, therefore it ended up with the enhancement of cell resistant. Although its resistance, bacteria growth was inhibited by the presence of the essential oil, due to the penetration and disruption of the phenolic compounds to the lipid structure of the cell membrane of bacteria, by destroying their enzyme system (Hamzekhani et al., 2024).

3.3. Comparison of caffeic acid and BLLEO as an active agent

Overall, comparing the effect of two active agent addition showed that there was no significant difference in the viscosity values of spinning solutions, both showing Newtonian behaviour. However, addition of BLLEO resulted in more viscous

solutions in comparison to the caffeic acid addition, as can be seen in Table 3.1 and Table 3.6. Similarly, although average fiber diameters did not change significantly with both active agents, BLLEO addition formed thicker fibers. These thicker fibers can be obtained due to the higher viscosity of solutions. In addition, the thinnest fiber diameters were obtained in control samples. The fiber morphology was not affected from the active agent addition and homogenous structure was preserved in both cases (Figure 3.2 & Figure 3.11).

TPC, AOA and EE results of fibers had the similar trends. The increment of active agent enhanced the TPC and AOA of fibers, whereas decrement was observed in EE. However, addition of caffeic acid showed higher TPC and AOA comparing to BLLEO.

The same ratios of active agents, 3%, resulted in 26.88 ± 0.32 and 1.99 ± 0.08 mg GAE/g for caffeic acid and BLLEO, respectively. Likewise, AOA results were 84.95 ± 1.06 and 18.10 ± 0.01% for 3% caffeic acid and BLLEO addition, respectively. The lower values obtained with the addition of BLLEO might be caused by the easy evaporation of the essential oils. On the other hand, EE results were similar with the values of 84.30 ± 3.25 and 80.96 ± 0.02% for 3% caffeic acid and BLLEO addition, respectively, indicating the successful and efficient encapsulation by centrifugal spinning method.

The effect of active agent addition on WVP results showed the same trend, which can be explained as the presence of active agent increased the WVP, however increasing the caffeic acid and BLLEO concentration did not change the results significantly.

According to the FTIR analysis, both caffeic acid and BLLEO added fibers showed similar peaks at the wavelength range of 1200 and 1700 cm^{-1}, indicating Amide I, Amide II and Amide III bands corresponding to the gelatin structure. Different from the caffeic acid added fibers, there were remarkable peaks at 2856, 2929, 1053 and 1373 cm^{-1}, belonging to BLLEO. On the other hand, caffeic acid exhibited peaks from 500 to 1500 cm^{-1}, and from 3200 to 3400 cm^{-1}. It is worth saying that the characteristic peak intensity of active agents enhanced with the addition of both caffeic acid and BLLEO. TGA results exhibited a final weight approximately 25% and 21% for caffeic acid and BLLEO added fibers, respectively.

Lipid oxidation results on olive oil indicated that increment of the active agents enhanced the prevention of lipid oxidation. TOTOX values were found as 27.980 ± 1.580, 17.887 ± 0.935 and 15.390 ± 0.804 for G-C, G-2CA and G-3CA, respectively. On the other hand, the results of control, BLLEO-1, BLLEO-3 and BLLEO-5 were 34.473 ± 2.208, 33.578 ± 1.436, 22.758 ± 3.193 and 21.478, respectively. According to that, TOTOX values, indicating the overall lipid oxidation, was lowered in olive oil samples with caffeic acid added fibers more than with BLLEO added ones. At the 14^{th} day, fibers with 2% and 3% caffeic acid addition had a reduction of 36% and 45%, respectively, as compared to the control sample. However, addition of 1%, 3% and 5%

BLLEO lowered the TOTOX values 3%, 34% and 38%, respectively. This finding was in line with the TPC and AOA results, which caffeic acid added fibers had higher values than BLLEO added ones. Antimicrobial activity of caffeic acid added fibers did not show any effect on microbial growth, however fibers with BLLEO inhibited the growth of *S. aureus* and *E. coli*. Finally, it is recommended to use caffeic acid added centrifugally spun fibers by having higher antioxidant activity and prevent lipid oxidation more than BLLEO added ones.

CHAPTER 4

CONCLUSION

The aim of this study is to develop gelatin-based active packaging materials incorporated with caffeic acid and BLLEO by centrifugal spinning and to analyze the ability of fibers to inhibit oxidation of olive oil. In this study, caffeic acid and BLLEO loaded gelatin-based films were developed by centrifugal spinning successfully.

Caffeic acid and BLLEO addition did not affect viscosity of gelatin solutions and the diameter of fibers. According to FESEM images, all the samples had homogenous and bead free structure. WVP values increased with the addition of both caffeic acid and BLLEO, but they were not affected by enhanced concentrations of neither caffeic acid nor BLLEO. The lowest WVP value belonged to control sample, which had no active agent inside. Fibers with antioxidant activity were produced successfully for both active agents, however AOA and TPC values of caffeic acid added fibers were higher than BLLEO added ones. The highest AOA and TPC values were obtained in the sample with 3% caffeic acid addition. Although AOA and TPC values increased by the addition of caffeic acid and BLLEO, EE values were affected adversely. The application of fibers showed a remarkable prevention on the formation of primary oxidation products (peroxides) and secondary oxidation products such as malondialdehyde, 2-alkenals and 2,4-dienals. All concentrations of caffeic acid and BLLEO reduced lipid oxidation, but the highest reduction was seen in G-3CA and BLLEO-5, respectively. Antimicrobial activity of fibers with BLLEO was more effective on *S. aureus* than *E. coli*, showing an inhibition zone of nearly more than two times for the fiber including 5% BLLEO.

As a result of optimization studies, thinner fibers were obtained with low gelatin and high BLLEO concentration at high flow rates. On the other hand, lower flow rates and high gelatin concentration resulted in lower AOA and TPC values. Therefore, the optimum condition for BLLEO added fibers was selected as the sample obtained by 20% gelatin concentration and 5% BLLEO concentration at 20 mL/h flow rate.

As a result, caffeic acid and BLLEO loaded centrifugally spun gelatin fibers can be recommended to be used as active packaging to prevent oxidation of foods. In this aspect, caffeic acid was selected as the active agent with its higher prevention from oxidation as compared to BLLEO. These fibers being biodegradable and environmentally friendly could be a promising material to be used in food packaging area.

For further studies, centrifugally spun fibers with different active agents such as gallic acid, curcumin, and plant extracts could be encapsulated to produce active food packaging. In addition, it is recommended to enhance mechanical properties of fibers by using crosslinking methods in order to use them as a real-life packaging material for foods such as nuts and meat products. Other polymers such as polyethylene oxide, gums and chitosan or their blends can be used to improve the mechanical properties of centrifugally spun fibers.

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APPENDICES

A. ANOVA TABLES

Table A. 1 One way Analysis of Variance (ANOVA) and Tukey's comparison test for fiber forming solutions containing different concentrations of caffeic acid

One-way ANOVA: Viscosity versus solutions

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor		3 control; G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.004489	0.002245	9.65	0.049
Error	3	0.000698	0.000233		
Total	5	0.005187			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0152534	86.54%	77.57%	46.18%

Means

Factor	N	Mean	St Dev	95% CI
control	2	0.32300	0.00566	(0.28867; 0.35733)
G-2CA	2	0.3140	0.0255	(0.2797; 0.3483)
G-3CA	2	0.26100	0.00424	(0.22667; 0.29533)

Pooled St Dev = 0.0152534

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean Grouping
control	2	0.32300 A
G-2CA	2	0.3140 A
G-3CA	2	0.26100 A

Means that do not share a letter are significantly different.

Table A. 2 One way Analysis of Variance (ANOVA) and Tukey's comparison test for diameters of fibers containing different concentrations of caffeic acid

One-way ANOVA: Fiber diameters versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	control; G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	438480	219240	0.83	0.436
Error	297	78253466	263480		
Total	299	78691946			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
513.303	0.56%	0.00%	0.00%

Means

Factor	N	Mean	St Dev	95% CI
control	100	1789.3	538.0	(1688.3; 1890.3)
G-2CA	100	1839.9	502.0	(1738.9; 1941.0)
G-3CA	100	1882.8	499.0	(1781.8; 1983.9)

Pooled St Dev = 513.303

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
G-3CA	100	1882.8	A
G-2CA	100	1839.9	A
control	100	1789.3	A

Means that do not share a letter are significantly different.

Table A. 3 One way Analysis of Variance (ANOVA) and Tukey's comparison test for water vapor permeability (WVP) values fibers containing different concentrations of caffeic acid

One-way ANOVA: WVP versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	control; G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	2.8736	1.43679	23.99	0.014
Error	3	0.1796	0.05988		
Total	5	3.0532			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.244705	94.12%	90.19%	76.47%

Means

Factor	N	Mean	St Dev	95% CI
control	2	2.7700	0.0509	(2.2193; 3.3207)
G-2CA	2	4.323	0.177	(3.772; 4.874)
G-3CA	2	4.135	0.382	(3.584; 4.686)

Pooled St Dev = 0.244705

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
G-2CA	2	4.323	A
G-3CA	2	4.134	A
control	2	2.7700	B

Means that do not share a letter are significantly different.

Table A. 4 One way Analysis of Variance (ANOVA) and Tukey's comparison test for total phenolic content (TPC) values of fibers containing different concentrations of caffeic acid

One-way ANOVA: TPC versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
concentration	2	G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
concentration	1	31.0266	31.0266	104.86	0.009
Error	2	0.5918	0.2959		
Total	3	31.6184			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.543961	98.13%	97.19%	92.51%

Means

concentration	N	Mean	St Dev	95% CI
G-2CA	2	21.313	0.702	(19.658; 22.968)
G-3CA	2	26.883	0.315	(25.228; 28.538)

Pooled St Dev = 0.543961

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

concentration	N	Mean	Grouping
G-3CA	2	26.883	A
G-2CA	2	21.313	B

Means that do not share a letter are significantly different.

Table A. 5 One way Analysis of Variance (ANOVA) and Tukey's comparison test for antioxidant activity (AOA) values of fibers containing different concentrations of caffeic acid

One-way ANOVA: AOA versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	2	G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	167.703	167.703	107.33	0.009
Error	2	3.125	1.563		
Total	3	170.828			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.25	98.17%	97.26%	92.68%

Means

Factor	N	Mean	St Dev	95% CI
G-2CA	2	72.00	1.41	(68.20; 75.80)
G-3CA	2	84.950	1.061	(81.147; 88.753)

Pooled St Dev = 1.25

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
G-3CA	2	84.950	A
G-2CA	2	72.00	B

Means that do not share a letter are significantly different.

Table A. 6 One way Analysis of Variance (ANOVA) and Tukey's comparison test for encapsulation efficiency (EE) values of fibers containing different concentrations of caffeic acid

One-way ANOVA: EE versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	2	G-2CA; G-3CA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	129.96	129.96	12.83	0.070
Error	2	20.26	10.13		
Total	3	150.22			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.18277	86.51%	79.77%	46.05%

Means

Factor	N	Mean	St Dev	95% CI
G-2CA	2	95.70	3.11	(86.02; 105.38)
G-3CA	2	84.30	3.25	(74.62; 93.98)

Pooled St Dev = 3.18277

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
G-2CA	2	95.70	A
G-3CA	2	84.30	A

Means that do not share a letter are significantly different.

Table A. 7 One way Analysis of Variance (ANOVA) and Tukey's comparison test for viscosity values of fiber forming solutions containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: Viscosity versus solutions

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	4	control; BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	3	0.004021	0.001340	0.48	0.716
Error	4	0.011282	0.002820		
Total	7	0.015303			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0531084	26.28%	0.00%	0.00%

Means

C1	N	Mean	St Dev	95% CI
control	2	0.4232	0.1036	(0.3189; 0.5275)
BLLEO-1	2	0.3760	0.0190	(0.2718; 0.4803)
BLLEO-3	2	0.42446	0.01269	(0.32020; 0.52873)
BLLEO-5	2	0.43339	0.00507	(0.32912; 0.53765)

Pooled St Dev = 0.0531084

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
BLLEO-5	2	0.43339	A
BLLEO-3	2	0.42446	A
control	2	0.4232	A
BLLEO-1	2	0.3760	A

Means that do not share a letter are significantly different.

Table A. 8 One way Analysis of Variance (ANOVA) and Tukey's comparison test for diameters of fibers containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: Fiber diameters versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	4	control; BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	37709809	12569936	35.94	0.000
Error	395	138157483	349766		
Total	398	175867292			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
591.410	21.44%	20.85%	19.84%

Means

Factor	N	Mean	St Dev	95% CI
Control	100	1541.4	103.9	(1425.2; 1657.7)
BLLEO-1	100	2259.9	21.0	(2143.1; 2376.8)
BLLEO-3	100	2315.0	361.0	(2198.7; 2431.3)
BLLEO-5	100	1956.4	11.2	(1839.9; 2072.4)

Pooled St Dev = 591.410

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
BLLEO-3	100	2315.0	A
BLLEO-1	100	2259.9	A
BLLEO-5	100	1956.4	A
Control	100	1541.4	A

Means that do not share a letter are significantly different.

Table A. 9 One way Analysis of Variance (ANOVA) and Tukey's comparison test for water vapor permeability (WVP) values fibers containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: WVP versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
C1	4	control; BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	3	5.0279	1.6760	9.58	0.027
Error	4	0.6996	0.1749		
Total	7	5.7275			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.418199	87.79%	78.63%	51.14%

Means

C1	N	Mean	St Dev	95% CI
control	2	4.2647	0.1187	(3.4437; 5.0858)
BLLEO-1	2	6.019	0.539	(5.198; 6.840)
BLLEO-3	2	6.033	0.415	(5.212; 6.854)
BLLEO-5	2	6.210	0.472	(5.389; 7.031)

Pooled St Dev = 0.418199

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C1	N	Mean	Grouping
BLLEO-5	2	6.210	A
BLLEO-3	2	6.033	A
BLLEO-1	2	6.019	A
control	2	4.2647	B

Means that do not share a letter are significantly different.

Table A. 10 One way Analysis of Variance (ANOVA) and Tukey's comparison test for antioxidant activity (AOA) values of fibers containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: AOA versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	117.185	58.592	30.40	0.010
Error	3	5.782	1.927		
Total	5	122.966			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.38826	95.30%	92.16%	81.19%

Means

Factor	N	Mean	St Dev	95% CI
BLLEO-1	2	12.9800	0.0424	(9.8560; 16.1040)
BLLEO-3	2	18.10	0.00	(14.98; 21.22)
BLLEO-5	2	23.80	2.40	(20.68; 26.92)

Pooled St Dev = 1.38826

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
BLLEO-5	2	23.80	A	
BLLEO-3	2	18.10	A	B
BLLEO-1	2	12.9800		B

Means that do not share a letter are significantly different.

Table A. 11 One way Analysis of Variance (ANOVA) and Tukey's comparison test for total phenolic content (TPC) values of fibers containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: TPC versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.39430	0.197150	34.59	0.008
Error	3	0.01710	0.005700		
Total	5	0.41140			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0754983	95.84%	93.07%	83.37%

Means

Factor	N	Mean	St Dev	95% CI
BLLEO-1	2	1.6200	0.0100	(1.4501; 1.7899)
BLLEO-3	2	1.9850	0.0778	(1.8151; 2.1549)
BLLEO-5	2	2.2450	0.0778	(2.0751; 2.4149)

Pooled St Dev = 0.0754983

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
BLLEO-5	2	2.2450	A
BLLEO-3	2	1.9850	A
BLLEO-1	2	1.6200	B

Means that do not share a letter are significantly different.

Table A. 12 One way Analysis of Variance (ANOVA) and Tukey's comparison test for encapsulation efficiency (EE) values of fibers containing different concentrations of bay laurel leaf essential oil

One-way ANOVA: EE versus fibers

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1656.95	828.477	758.32	0.000
Error	3	3.28	1.093		
Total	5	1660.23			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.04524	99.80%	99.67%	99.21%

Means

Factor	N	Mean	St Dev	95% CI
BLLEO-1	2	94.77	1.44	(92.41; 97.12)
BLLEO-3	2	80.9625	0.0177	(78.6104; 83.3146)
BLLEO-5	2	54.700	1.103	(52.348; 57.052)

Pooled St Dev = 1.04524

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
BLLEO-1	2	94.77	A
BLLEO-3	2	80.9625	B
BLLEO-5	2	54.700	C

Means that do not share a letter are significantly different.

Table A. 13 One way Analysis of Variance (ANOVA) and Tukey's comparison test for antimicrobial activity values of fibers containing different concentrations of bay laurel leaf essential oil on E. coli

One-way ANOVA: area (%) versus sample *E. coli*

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
sample	3	BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	2	1.6357	0.81785	20.34	0.018
Error	3	0.1206	0.04021		
Total	5	1.7563			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.200534	93.13%	88.55%	72.52%

Means

sample	N	Mean	St Dev	95% CI
BLLEO-1	2	1.6420	0.1216	(1.1907; 2.0933)
BLLEO-3	2	2.0110	0.0311	(1.5597; 2.4623)
BLLEO-5	2	2.887	0.324	(2.436; 3.338)

Pooled St Dev = 0.200534

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping
BLLEO-5	2	2.887	A
BLLEO-3	2	2.0110	B
BLLEO-1	2	1.6420	B

Means that do not share a letter are significantly different.

Table A. 14 One way Analysis of Variance (ANOVA) and Tukey's comparison test for antimicrobial activity values of fibers containing different concentrations of bay laurel leaf essential oil on S. aureus

One-way ANOVA: area (%) versus sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
sample	3	BLLEO-1; BLLEO-3; BLLEO-5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	2	39.945	19.9723	24.25	0.014
Error	3	2.471	0.8237		
Total	5	42.416			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.907558	94.17%	90.29%	76.70%

Means

sample	N	Mean	St Dev	95% CI
BLLEO-1	2	2.014	0.530	(-0.028; 4.056)
BLLEO-3	2	7.009	0.187	(4.967; 9.051)
BLLEO-5	2	7.87	1.47	(5.82; 9.91)

Pooled St Dev = 0.907558

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping
BLLEO-5	2	7.87	A
BLLEO-3	2	7.009	A
BLLEO-1	2	2.014	B

Means that do not share a letter are significantly different.

B. CALIBRATION CURVES

Figure B. 1 Calibration curve for total phenolic content by using gallic acid

