

THE EFFECTS OF CITRIC ACID CROSSLINKING ON FABRICATION AND
CHARACTERIZATION OF GELATIN/CURCUMIN BASED ELECTROSPUN
ANTIOXIDANT NANOFIBERS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

REEM HASAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

SEPTEMBER 2023

Approval of the thesis:

**THE EFFECTS OF CITRIC ACID CROSSLINKING ON FABRICATION
AND CHARACTERIZATION OF GELATIN/CURCUMIN BASED
ELECTROSPUN ANTIOXIDANT NANOFIBERS**

submitted by **REEM HASAN** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Hami Alpas
Head of the Department, **Food Engineering**

Prof. Dr. Servet Gülüm Şumnu
Supervisor, **Food Engineering, METU**

Prof. Dr. Serpil Şahin
Co-Supervisor, **Food Engineering, METU**

Examining Committee Members:

Prof. Dr. Mecit Halil Öztop
Food Engineering, METU

Prof. Dr. Servet Gülüm Şumnu
Food Engineering, METU

Assist. Prof. Dr. Elif Yolaçaner
Food Engineering, Hacettepe University

Assist. Prof. Dr. Leyla N. Kahyaoğlu
Food Engineering, METU.

Assist. Prof. Dr. Nalan Yazıcıoğlu
Nutrition and Dietetics, University of Health Sciences

Date: 01.09.2023

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name : Reem Hasan

Signature :

ABSTRACT

THE EFFECTS OF CITRIC ACID CROSSLINKING ON FABRICATION AND CHARACTERIZATION OF GELATIN/CURCUMIN BASED ELECTROSPUN ANTIOXIDANT NANOFIBERS

Hasan, Reem
Master of Science, Food Engineering
Supervisor : Prof. Servet Gülüm Şumnu
Co-Supervisor: Assoc. Prof. Dr. Serpil Şahin

September 2023, 92 pages

Electrospinning method is in high demand in these days due to its ability to produce nanofibers which have different optical, electrical, thermal, and mechanical properties than macro scale materials. In this study, nanofibers were produced through electrospinning using gelatin as the base polymer and curcumin as the functional agent. The effects of crosslinking with citric acid (0.5% and 1.0% w/v) were studied along with the effect of gelatin concentration on the characteristics of electrospun nanofibers. Neat gelatin film was used as control. Different characterization tests were performed on the solutions, including solution conductivity, color analysis and rheological properties, while the obtained nanofibers were characterized by morphological analysis (SEM), Antioxidant capacity (AA), Total Phenolic Content (TPC), Encapsulation Efficiency (EE), thermal properties (TGA, XRD, DSC), Water Vapor Permeability (WVP), Fourier Transform Infrared Analysis (FTIR) and Antimicrobial Activity. The results showed that some degree of crosslinking was seen in the fibers, that was indicated by the changes in AA, and crystallinity results. The highest antioxidant capacity seen was for gelatin and

curcumin films containing no citric acid (32%). The highest melting temperature (78°C) and WVP ($2.365 \times 10^{-10} \text{ gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$) were seen for gelatin and curcumin films crosslinked with 0.5% citric acid, while the lowest crystallinity (1.56%) was seen for gelatin with curcumin films crosslinked with 1% citric acid. This study showed that even though citric acid might not prove to be a stable crosslinking agent for the protein (gelatin), it contributed to the antioxidant nature of the films, along with curcumin. These films are promising candidates to be applied on packaging for cut fruits, to reduce their water loss and oxidation and hence to extend their shelf lives.

Keywords: Electrospinning, Crosslinking, Curcumin, Phenolics, Nanofibers

ÖZ

SİTRİK ASİT ÇAPRAZ BAĞLAMININ JELATİN/KURKUMİN BAZLI ELEKTROSPUN ANTİOKSİDAN NANOLİFLERİN ÜRETİMİ VE KARAKTERİZASYONU ÜZERİNDEKİ ETKİLERİ

Hasan, Reem
Yüksek Lisans, Gıda Mühendisliği
Tez Yöneticisi: Prof. Dr. Servet Gülüm Şumnu
Ortak Tez Yöneticisi: Prof. Dr. Serpil Şahin

Eylül 2023, 92 sayfa

Elektroegirme yöntemi, makro ölçekten farklı optik, elektriksel, termal ve mekanik özelliklere sahip olan nanolif üretme özelliği nedeniyle bugünlerde çok talep görmektedir. Bu çalışmada, polimer olarak jelatin ve fonksiyonel ajan olarak kurkumin kullanılarak elektroegirme yöntemiyle nanolifler üretilmiştir. Jelatin konsantrasyonunun ve sitrik asit (%0,5 ve %1,0 w/v) ile çapraz bağlamanın elektroegirilmiş nanoliflerin özellikleri üzerindeki etkileri çalışılmıştır. Sadece jelatin içeren film kontrol olarak kullanılmıştır. Çözeltilerin iletkenlik, renk analizi ve reolojik özellikleri ölçülürken, elde edilen nanolifler morfolojik analiz (SEM), Antioksidan Aktivite (AA), Toplam Fenolik İçerik (TPC), Kapsülleme Verimliliği (EE), termal özellikler (TGA, XRD, DSC), Su Buharı Geçirgenliği (WVP), Fourier Dönüşümü Kızılötesi Analizi (FTIR) ve Antimikrobiyal Aktivite ile karakterize edilmiştir. AA ve, kristallik analiz sonuçlarına göre bir dereceye kadar çapraz bağlanmanın olduğu gösterilmiştir. Sitrik asit içermeyen jelatin ve kurkumin filmlerinde en yüksek antioksidan aktivite görülmüştür (%32). En yüksek erime sıcaklığı (78°C) ve WVP ($2,365 \times 10^{-10} \text{ gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$) %0,5 sitrik asit ile çapraz

bağlanmış jelatin ve kurkumin filmlerinde görülürken, en düşük kristallik %1,0 sitrik asit ile çapraz bağlanmış kurkumin filmlerinde görülmüştür. Bu çalışma, sitrik asitin protein (jelatin) için kararlı bir çapraz bağlama maddesi olmadığını kanıtlasa da, kurkumin ile birlikte filmlere antioksidan özellik kazandırılmıştır. Bu filmler, su kaybını ve oksidasyonu azaltmak ve dolayısıyla raf ömürlerini uzatmak için kesilmiş meyvelerin ambalajlarında kullanılmaya adaydır.

Anahtar Kelimeler: Elektroğirme, Çapraz Bağlama, Kurkumin, Fenolikler, Nanolifler

To my beloved family.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Gülüm Şumnu, for providing me with this project as my thesis and for always guiding me towards the right research methods. The experience I have gained in the past two years would not have been possible without her. I would also like to thank Prof. Dr. Serpil Şahin for her valuable opinions and insights on my study.

I thank everyone at the Food Engineering department who helped me understand everything as a new lab member and international student. To my fellow lab members Eda Yıldız, Demet Sönmezler, Emre Kızıl, and Güneş Su Güler, for helping me understand my research through many angles and teaching me any analysis or procedure I was not clear on. I also thank members and assistants of other labs for taking out time to help me understand usage of certain equipment, without which I could not have completed many of the characterization analysis.

I thank all staff members, faculty and students of Food Engineering, METU, for greeting me with a kind smile every day.

My special thanks goes to my husband, Abdul Ahad Khan, for his extremely patient and positive attitude, for always providing me with a listening ear, for giving me motivation throughout my thesis journey and space when I needed it, for making me laugh on my bad days and for helping me balance my personal and academic life. It would have been manifold harder without his support.

Lastly, I would like to thank my family. Even though we are divided into 3 different countries, a short phone call every day has helped me stay connected with myself and them and has helped me unwind daily. I thank them for providing me with logical suggestions, prayers, motivation and for trying their best to understand my nature of work.

TABLE OF CONTENTS

ABSTRACT.....	v
ÖZ.....	vii
ACKNOWLEDGMENTS	x
TABLE OF CONTENTS.....	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvi
1 INTRODUCTION	1
1.1 Sustainable and active packaging	1
1.2 Electrospinning	3
1.2.1 Process and equipment.....	5
1.2.2 Parameters affecting electrospinning	6
1.2.3 Advantages of electrospinning technique	10
1.3 Gelatin as a base polymer	10
1.4 Curcumin as a functional agent.....	12
1.5 Crosslinking	14
1.5.1 Glutaraldehyde crosslinking	14
1.5.2 Citric acid crosslinking	15
1.6 Objectives and novelty of the study	16
2 MATERIALS AND METHODS.....	19
2.1 Materials	19
2.2 Solution Preparation.....	19

2.3	Solution Properties	20
2.3.1	Rheological Properties.....	20
2.3.2	Color.....	20
2.3.3	Electrical Conductivity	21
2.4	Electrospinning.....	21
2.5	Nanofiber film characterization.....	21
2.5.1	Morphological Analysis	22
2.5.2	Color.....	22
2.5.3	Antioxidant Capacity.....	22
2.5.4	Total Phenolic Content.....	23
2.5.5	Encapsulation Efficiency.....	23
2.5.6	Thermogravimetric Analysis	24
2.5.7	Differential Scanning Calorimetry	24
2.5.8	X-ray Diffraction	24
2.5.9	Thickness of film.....	25
2.5.10	Water Vapor Permeability	25
2.5.11	Fourier Transform Infrared (FTIR) Analysis.....	26
2.5.12	Antimicrobial Analysis	26
2.6	Statistical Analysis	26
3	RESULTS AND DISCUSSION.....	29
3.1	Solution properties, fiber morphology and color	29
3.2	Antioxidant capacity.....	35
3.3	Total Phenolic Content and Encapsulation Efficiency	36
3.4	Thermogravimetric analysis	37

3.5	Differential Scanning Calorimetry	39
3.6	Crystallinity Analysis (XRD)	42
3.7	Water Vapor Permeability	43
3.8	Fourier Transform Infrared Analysis (FTIR).....	44
3.9	Antimicrobial Activity	45
4	CONCLUSION AND RECOMMENDATIONS	47
	REFERENCES	49
5	APPENDICES	67
A.	Statistical Analysis	67
B.	Calibration Curves	91

LIST OF TABLES

TABLES

Table 2.1 Nomenclature of solutions and their respective films	19
Table 3.1 Solution properties of polymer solutions and their respective fibre diameters.....	30
Table 3.2. Color results (L*, a* and b* values) of the solution and the films.....	34
Table 3.3 Antioxidant capacity of films	35
Table 3.4 Total Phenolic content and Encapsulation Efficiency of films	37
Table 3.5 Two stage weight loss percentage and crystallinity percentage of films	38
Table 3.6 Glass transition (Tg), Melting (Tm) and Degradation Temperatures (Tdeg) of films.....	41
Table 3.7 Water Vapor Permeability and thickness of films.....	44

LIST OF FIGURES

Figure 1.1 Representation of an electrospinning apparatus. Reprinted from "Electrospun nanofibers: solving global issues," by. Ramakrishna et al., 2006, Materials Today, 9(3), 40–50. https://doi.org/10.1016/S1369-7021(06)71389-X ...	6
Figure 3.1 Nanofiber morphology for different gelatin concentrations (a) 20%GL, (b) 25%GL, (c) 30%GL, (d) 35%GL	31
Figure 3.2 SEM images and fiber size distributions: (a) GL (b) GLC (c) GLCCA0.5 (d) GLCCA1	33
Figure 3.3 TGA weight loss graphs	38
Figure 3.4 XRD graphs of films	42
Figure 3.5 FTIR spectra of films.....	45
Figure 3.6 Antimicrobial analysis (a) GLCCA0.5 (b) GLCCA1.....	46

LIST OF ABBREVIATIONS

ABBREVIATIONS

GL - Gelatin

C - Curcumin

CA – Citric Acid

AA – Antioxidant capacity

TPC – Total Phenolic Content

EE – Encapsulation Efficiency

TGA – Thermogravimetric Analysis

DSC – Differential Scanning Calorimetry

XRD – X-ray Diffraction

FTIR – Fourier Transform Infrared Analysis

WVP - Water Vapor Permeability

CHAPTER 1

INTRODUCTION

In the recent past, the food industry all over the world has been seeing revolutionary changes as part of its goal for sustainable development of food and agriculture. A lot of focus is being given to production of packaging materials that help to increase the shelf life of foods while being more environmentally friendly and cost-effective. The use of nanofibers produced from substances like gelatin is being researched upon, to assess the possibility of encapsulating antimicrobial, antibacterial and antioxidative agents in them to extend their shelf-lives. To keep foods fresh for a longer time, a sustainable film must be developed. A lot of factors need to be considered, such as toxicity of the polymer, rheological properties, mechanical strength, gelation temperature etc.

1.1 Sustainable and active packaging

There has been a growing need for better solutions of packaging materials in terms of the materials used, disposal and impact on pollution, in order to protect our environment and at the same time to provide a longer shelf life of foods. There is demand for healthier and safer foods and for that, the packaging needs to be non-toxic, biodegradable, environmentally friendly and keep our foods fresh for longer. They must also be socially and economically viable and require minimum energy and materials to be produced (Russell, 2014). The four most common materials used for packaging are plastic, glass, metal and paper.

Among the many materials used for packaging, we have plastics, which, although quite useful for food packaging in terms of their light weight, flexibility, strength and transparency (Siracusa & Rosa, 2018), pose a threat in terms of being non-

biodegradable and thereby increase the environmental footprint. Glass is one packaging material that is easily recyclable and also attractive to consumers, but it weighs a lot and can be easily broken upon physical or thermal stress (Ibrahim et al., 2022). Another very commonly used packaging material is metal. The kind of metal being used is very important because metal has the tendency to leak into or react with the food item and cause toxicity, therefore assessment of safety when interacting with the food in question is important before using any metal (Ibrahim et al., 2022).

Lastly, the most superior form of packaging is paper and paperboard, which, although biodegradable and recyclable, can be easily susceptible to moisture. This can be overcome with coating of polymers onto the paper such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), and polyester (PET) (Walsh & Kerry, 2012). However, there is still a need for all of these materials to be made more safe for human consumption as well as the environment and therefore new forms of packaging such as vacuum packaging, modified atmosphere packaging, intelligent packaging and active packaging are being explored.

One of these kinds of packaging materials, known as active packaging, refers to a packaging material that can either release components to fight any pathogens or absorb these harmful pathogens – in short, a packaging material with the ability to eliminate any substance that can potentially harm the food (Yildirim et al., 2017). Some forms of active scavenging packaging materials are those that are oxygen scavengers, moisture scavengers and/or ethylene absorbers, while active releasing materials include those that are antioxidant in nature, carbon dioxide emitters and/or antimicrobial. These packaging materials can be used for a wide variety of foods, including dairy, meat, fresh fruits and vegetables, nuts and many more (Yildirim et al., 2017). Until now, antioxidant nature of packaging materials was obtained through synthetic substances such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), but now there is an increasing interest towards incorporating more natural ingredients such as polyphenols, plants extracts and essential oils

(Barbosa-Pereira et al., 2014; Marcos et al., 2014). Some examples of natural antioxidant compounds are green tea extract (catechin), which was seen to slow down the oxidation of sunflower oil and fried peanuts (López-De-Dicastillo et al., 2012), thymol, carvacrol, and eugenol which improved stability of beef patties during storage by inhibiting lipid oxidation (Park et al., 2012), as well as oregano and rosemary extract, which were also used to successfully prevent oxidation of lamb meat (Camo et al., 2008). Apart from this, curcumin also proves to be an important antioxidant polyphenol. Curcumin has many anti-inflammatory and antioxidant properties and it can be used as a pH indicator to monitor the quality of foods; it can be used as a biosensor in both smart and active packaging (Oliveira Filho et al., 2021). It has shown to positively affect delaying of chia oil degradation when it was added in TPCS/PBAT films, where it conferred antimicrobial activity against both Gram-positive and Gram-negative bacteria and also showed better antioxidant capacity (Silva De Campos et al., 2019). Curcumin was also incorporated into soybean polysaccharide and gelatin films as active packaging for soybean oil, and it showed promising results through better structural properties, antioxidant capacity, scavenging of *Escherichia coli* and *Staphylococcus aureus*, and delaying soybean oil oxidation (Dong et al., 2023). While there are other methods of scavenging oxygen such as vacuum packaging and modified atmosphere packaging, these packaging types are not completely effective and therefore active packaging wins over (Alves et al., 2022).

1.2 Electrospinning

Electrospinning is a novel method of producing homogenous micro or nanofibers where the polymer solution is stretched under the effect of an electric field (Aydogdu, Sumnu, et al., 2019). The use of these fibers is more common in the cosmetics, pharmaceutical and biomedical industries, but it is an almost uncharted territory in the food industry yet. Producing nanofibers using the method of electrospinning has shown to be an effective method for drug delivery in many cases.

With electrospinning, the bioavailability of drugs can be improved as many drugs can be loaded at one time into the solution, and hence fibers, and controlled release of substances can be achieved (Vass et al., 2020). These drugs can range from being antibiotics, wound dressing, anti-cancer, anti-inflammatory or anti-fungal drugs, among many other drug categories, and a similar concept is being delved into in the food industry so that certain desired properties can be more efficiently put into effect, especially in packaging products. Electrospun nanofibers have also been studied upon for cosmetic products, such as encapsulation of fatty oils with antioxidant properties using pomegranate and sea-buckthorn (Miletić et al., 2019). In this study, active compounds from these were encapsulated into polylactide (PLA-based fibers) and poly(vinyl-pirrolidone) (PVP-based fibers) nanofibers using the method of electrospinning and various properties such as thermal, mechanical, as well as uniformity of the fibers was tested to assess the potential for use in the cosmetics industry, and it was seen that PLA-based films proved to have better antioxidant properties than PVP-based films. The combination of basic polymers as well as bioactive compounds needs to be experimented upon to find the optimal concentration, combination and characteristics in any nanofibers produced by assessing properties such as mechanical strength, thermal stability, crystallinity etc. These fibers can have many unique characteristics, including being highly porous and having a larger surface area (Zhao et al., 2020), apart from the ability to hold encapsulated substances to improve its bioavailability for functionality (de Dicastillo et al., 2019). When attached to a plastic sheet, these nanofibers can serve as packaging materials. Gallic acid loaded electro-spun nanofibers were fabricated as active packaging material to prevent walnuts from oxidation (Aydogdu, Sumnu, et al., 2019). These fibers proved to have high thermal stability (Aydogdu, Yildiz, et al., 2019), and the electrospinning method proved to be efficient in encapsulating bioactive compounds (gallic acid). The electrospinning technique was also applied in producing nanofibers using chickpea flour and polyethylene oxide (PEO), with curcumin incorporated as a functional agent (Yildiz, Sumnu, et al., 2022). Microwave heating was also applied on the solutions to assess its impact on these

nanofibers. The study showed that addition of curcumin improved the functionality of the films, with one of the highlights being the shift in onset degradation temperature by 20°C. Microwave heating also helped along with addition of curcumin to increase the antioxidant capacity, concluding the fact that microwave heating of solutions was a favorable method to apply along with electrospinning method. Gelatin-zein-glucose electro-spun nanofibers were also produced, with Maillard reaction occurring between the protein and glucose molecules and these films also showed greater thermal stability and hydrophobic surfaces (Liu et al., 2023). In addition to this, ferulic acid was used as an agent to improve the antioxidant nature of fibrous films produced with zein and polyethylene oxide, and improved the shelf-life of apples. This study showed promising effects of electrospinning on food application (Huang et al., 2022). Therefore, electrospun fibers can aid in preventing or delaying oxidation.

1.2.1 Process and equipment

Over the last few decades, there has been an increasing interest towards learning and implementation of electrospinning. The process and apparatus involved has shown to be far simpler and more cost-effective than other nanofiber building processes such as gas jet and melt fibrillation, apart from also being time-efficient. (Ramakrishna et al., 2006). Even though the idea of producing nanofibers using an electric field sounds complicated, it is in fact a simple procedure. The electrospinning machine, as shown in Figure 1.1, consists of a syringe with a pump attached to it that controls the flow rate, connected to one end of the electrode, and a collector plate, usually covered with aluminum foil, connected to the other electrode. As a voltage is provided and the solution is electrified, an electric field is created between the two electrodes as the solution is pumped out. After the electric field overcomes the surface tension of the solution, the solution stretches under the effect of the electric field and the droplet forms into a Taylor cone as a whipping motion is created due to bending instabilities (Xue et al., 2019). The stretching continues until the solution

deposits as fibers on the collector plate with fine diameters. In the process of travelling from the syringe to the collector plate, the solution solidifies as volatile matter escapes, and thus fibers are formed.

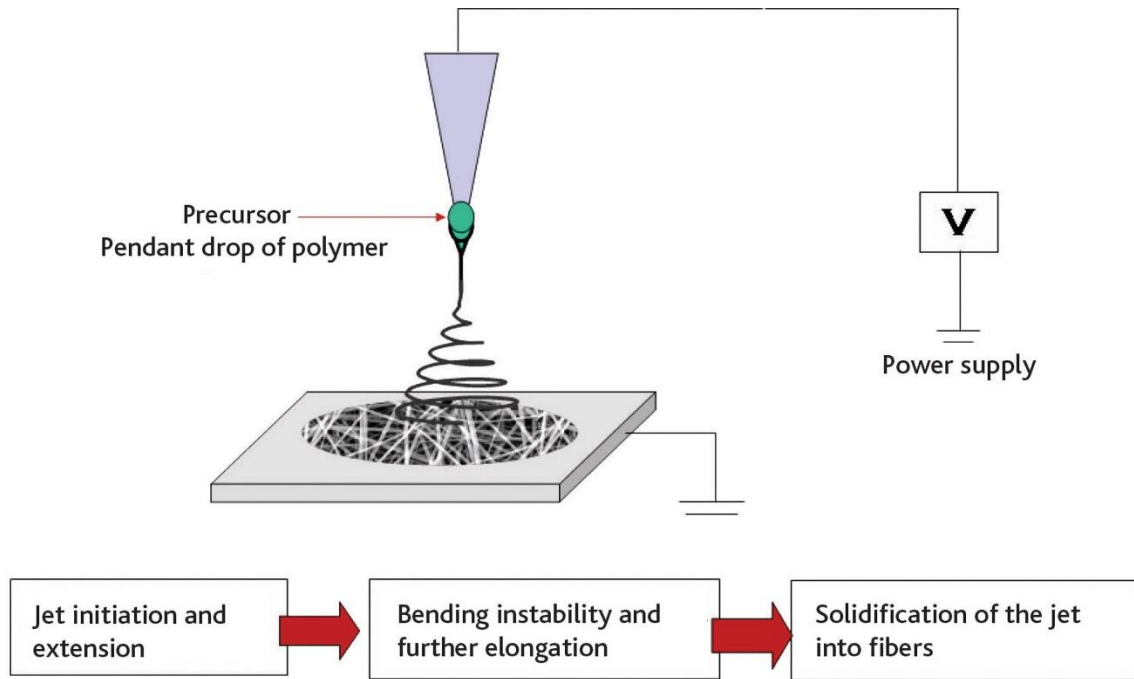


Figure 1.1 Representation of an electrospinning apparatus. Reprinted from "Electrospun nanofibers: solving global issues," by. Ramakrishna et al., 2006, *Materials Today*, 9(3), 40–50. [https://doi.org/10.1016/S1369-7021\(06\)71389-X](https://doi.org/10.1016/S1369-7021(06)71389-X)

1.2.2 Parameters affecting electrospinning

1.2.2.1 Solution parameters

It is important to be able to obtain thin, uniform and bead-less fibers through electrospinning, and many factors come into play for that to occur. First and foremost, come the solution parameters, which include viscosity, conductivity, surface tension and solvent choice.

When solutions have a low viscosity, there is a high chance for bead formation in the electrospun fibers and hence non-uniform fibers. (Robb & Lennox, 2011).

Viscosity can be altered by the weight of the polymer as well as temperature, and even when the weight of the polymer used is kept constant, it is also important to keep the temperature at which the solution is spun constant in order to be able to control the diameter and uniformity of fibers. It is known that an increased viscosity (with increased polymer concentration) leads to fewer beads in the fibers (Fong et al., 1999). However, too high viscosity can also lead to the inability of the solution to eject through the syringe. Apart from that, in cases where the nanofibers must be produced at a temperature above or below the optimal temperature for any solution, additives such as salts can be used to manipulate the viscosity (Du & Zhang, 2008).

Solution conductivity is also an important parameter in electrospinning. The solution being spun should be conductive (ionic in nature) so that the electric field may have an effect on it and it can flow between the syringe and collector plate. Solution conductivity can be changed by addition of salts, and a higher conductivity leads to thinner and more uniform fibers (Hsu & Shivkumar, 2004).

Surface tension also plays a major role in the production of desired nanofibers. The electric field in the machine needs to be able to overcome the surface tension the solution, for the solution to form a jet. Solutions with high surface tension tend to form beads as beads, since beads have a high volume to surface area ratio and through those, the surface energy of the material is lowered (Robb & Lennox, 2011). However, surface tension can be adjusted through addition of surfactants in case where the materials used cannot be changed. One of the most used surfactant, which was also a part of this study, is Tween 80 (Polysorbate 80). It is hydrophilic and non-ionic and is used as an emulsifier as well. In a study conducted on chitosan (CS) and polyvinyl alcohol (PVA) solution electrospinning, addition of Tween 80 showed to have a decreasing effect on the viscosity of the fibers forming solution, and improved mechanical properties and wettability of the nanofiber mats. (Boonpratum et al., 2022) It showed that it made the mats more hydrophilic and hence favored the drug release property of the mats.

Lastly, in terms of solution properties, the choice of solvent used also plays an important role in the properties of the nanofibers. The solvent needs to evaporate as the solution travels to the collector plate in the electrospinning machine, and hence more volatile solvents, such as alcohols are a good choice to use if they favor dissolution of the materials being used. In cases where more than one solvent is used, the polymer-containing solution can have varying viscosities throughout and as a result, the fibers formed may also have significant variation between them (Robb & Lennox, 2011).

1.2.2.2 Process parameters

The second category of parameters that affect the electrospinning process are the processing parameters, that include electric field strength, spinning distance, flow rate, humidity and temperature.

The electric field strength is the parameter that is responsible for overcoming the surface energy (surface tension) of the solution. Both an AC or DC supply is used in electrospinning machines and a voltage range between 5-35 kV is used most of the time (Robb & Lennox, 2011). Increasing the electric field strength above the optimal value required for any particular solution can have both a positive and negative effect on the fiber diameters. Although a higher voltage causes greater whipping instability and hence longer fiber jet elongation, it also means that the time it takes for the fibers to reach the collector plate decreases (Williams et al., 2021). This may cause little or no effect on actual thinning of the fibers.

The spinning distance is also linked to electric field strength, where a shorter distance means greater electric field strength between the two electrodes, but a short distance yet again means less flight time for the jet, which may result in ineffective elongation of the fibers to their desired thickness. Usually, a spinning distance of 100-200 mm is used, and it was shown in one study where polyurethane in tetrahydrofuran and DMF mixed solvents was spun, that when the spinning distance was increased from

10 to 30 cm, a steady increase in fiber diameter was observed, showing that a longer distance is not always effective in thinning the diameters (Kidoaki et al., 2006).

The flow rate refers to the rate at which the solution is fed through the pump into the syringe needle. The optimum range of flow rates varies between polymers used in the solution as well as the inner diameter of the needle, along with the voltage applied in the system (Williams et al., 2021). If the flow rate is beyond the optimum range, the diameters may increase as there would not be sufficient time given for jet elongation and Taylor cone formation.

1.2.2.3 Environmental parameters

Apart from the parameters mentioned above, relative humidity and temperature also contribute to changes in the fibre diameter. It is best to conduct electrospinning in an environment that is controlled in terms of relative humidity and temperature. The humidity within the electrospinning chamber is often controlled by silica gels, which help to keep the humidity between 30-40% at most times. A high humidity slows down the rate of solvent evaporation and hence the fibres get a longer time for elongation (Williams et al., 2021). However, if the relative humidity goes beyond a certain level, about 60%, then there are chances of more water absorption by the jet from the surroundings, especially with hygroscopic polymers, rather than solvent evaporation taking place.

Temperature also affects the way the jet of solution spins, in both a good and bad way. On one hand, it helps the molecules in solution have more energy and thereby decrease their viscosity and surface tension so that they have a better chance at elongating (Williams et al., 2021). On the other hand, a high temperature also means that the solution needs less time to evaporate, and this also means that the solution jet has less time to elongate, and thus thin fibre diameters may not be produced (Robb & Lennox, 2011). Hence, an optimum temperature is to be determined depending on polymer material, solvent type, and relative humidity.

1.2.3 Advantages of electrospinning technique

With the right set of parameters chosen for any solution being electrospun, there are many advantages of producing nanofibers through electrospinning in place of using other techniques such as melt blowing, bicomponent extrusion, phase separation and many others. Some of the biggest benefits of this method are its efficient and simple processing and cheap construction (Tam et al., 2017). It is a method that can produce non-woven films or mats and these films have a high volume/area ratio. This means that the nanofibers have a high surface area with small pore sizes, i.e., high porosity (El-Sakhawy & Elshakankery, 2017). Due to their small fiber diameters, they also have a low weight and high permeability, and can be used to incorporate active agents for functionality, whether they are being used in the food, chemical, or pharmaceutical industry. An example can be taken of carotene, an antioxidant compound that was encapsulated into zein nanofibers using electrospinning so that its stability against light and heat could be improved. It was seen that carotene retained its chemical structure better and did not decrease significantly after exposure to UV-visible radiation (Fernandez et al., 2009). Hence, in short, the use of these fibers is being explored more now because these fibers have many unique characteristics, including being highly porous and having a larger surface area (Zhao et al., 2020), apart from the ability to hold encapsulated substances to improve its bioavailability for functionality (de Dicastillo et al., 2019).

1.3 Gelatin as a base polymer

Gelatin is a polypeptide (protein) derived by hydrolyzing collagen (Etxabide et al., 2017) can be obtained from multiple sources; pigs (porcine gelatin), cow (bovine gelatin) and the heads, skins and, bones of fish. For packaging materials, bovine gelatin proves to be a logical choice due to its better mechanical properties and biocompatibility. Fish gelatin, although becoming increasingly popular, has the main drawback of being less stable to shear stresses and not having a competitive price

due to its recent development (Ramos et al., 2016). As far as porcine gelatin is concerned, it has the major drawback of being non-halal and non-kosher and hence provides ethical/religious concerns for a large group of consumers, especially in parts of the world such as Asia and Africa. Gelatin from cows has higher gelling and melting temperatures, which makes it a more suitable substance in several food applications (Ninan et al., 2014). It consists of carboxyl, hydroxyl, and amino groups as functional groups, which also serve as durable film-forming substances (Cai et al., 2019).

Gelatin is known to be soluble in water, and its solubility needs to be decreased to make it more compatible to be used to produce films for food packaging materials. Solubility may be reduced by adding many different types of polymers to make composite films, one of which is chitosan. Chitosan was incorporated into bovine gelatin and tuna fish gelatin films to see its effect on the physiochemical properties of films and in the presence of chitosan, the WVP of both types of films was seen to improve (Gómez-Estaca et al., 2010). The method of casting was used to produce composite films using waste gelatin (WG) from agricultural and industrial by-product. These films were either crosslinked with glutaraldehyde or blended with poly (vinyl alcohol) (PVA) and sugarcane bagasse (Chiellini et al., n.d.). WG films showed very fast biodegradability in soil simulating conditions, but this degradation rate slowed down as they were strengthened with glutaraldehyde or PVA. Shellac, a resin obtained from lac bugs, was also combined with gelatin in another study. The mechanical properties were seen to improve, including puncture strength and percentage elongation (Soradech et al., 2012). In another study which was aimed to produce calorimetric films as part of smart packaging applications, fish gelatin was used, along with UV light radiation and carbon dots (CD). Chicken breast samples were tested upon with these films and the colour difference of the films was seen to correlate with microbial growth and Total Volatile Basic Nitrogen (TVB-N) release (Kilic et al., 2022). There is comparison made between films formed through casting method and those formed through electrospinning, and electrospinning was seen to win in many cases. When gelatin and zein films were created using both casting and

electrospinning, the films formed through electrospinning showed better hydrogen bonding, dispersion of zein particles within the nanofibers and hydrophobicity (Deng et al., 2017). In a study conducted on electrospinning with gelatin solutions, different concentrations of gelatin (15%, 20%, 25% and 30%) were tested to see its effect on the chemical, morphological and degradation properties of the electrospun fibres (YIKAR et al., 2021). Other agents were mixed in to strengthen the films and to make them less soluble. It was seen that with an increase in gelatin concentration, an increase in fiber diameters was seen, as well as an increase in the pore size between fibres. The average fibre diameters ranged from 142.47 to 451.64 nm. With gravimetric analysis, the degradation properties of the nanofibers were also assessed and it was seen that films with a higher gelatin concentration showed lesser degradation and hence were more stable when incubated. Many different types of composite films with different functional agents are present but there are no studies present on gelatin/curcumin films crosslinked with citric acid to assess its applicaiton in the food industry.

1.4 Curcumin as a functional agent

Polyphenols are compounds that are found in plant-based foods and beverages and are responsible for many positive impacts on human health, including, but not limited to, protection against cardiovascular diseases and type 2 diabetes (Williamson, 2017). Curcumin, a polyphenol belonging to the family of curcuma (*Curcuma longa* L.), is most commonly found in turmeric, and is commonly used to flavor food and also as a dye (Martins et al., 2016). Turmeric (curcumin) is also used as an herbal supplement and in the cosmetics industry, apart from the food industry. It has remarkable nontoxic, anti-inflammatory, antioxidant, and antimicrobial properties (Hwang & Shin, 2018). In a study conducted by Bojorges, et al (2020), the antimicrobial and antioxidant effects of curcumin were discussed, and it was noted that it proved to be better antioxidant in comparison to herbal extracts such as those from seaweed. This proves its ability to preserve food items naturally. Curcumin was

also added in an active-intelligent packaging film made with pullulan/chitin nanofibers and anthocyanins, and films with curcumin showed better resistance to pH change (Duan et al., 2021). However, due to its low water solubility, it is often difficult to incorporate it into foods, packaging, or pharmaceutical products. Of the many solutions to this challenge, one is the method of encapsulation, which produces a delivery system that breaks down gradually with increase in storage time or effect of temperature (Dalla et al., 2022). In one study, curcumin was encapsulated in gelatin through electrohydrodynamic atomization, and its anti-microbial and antioxidant properties were assessed (Gómez-Estaca et al., 2017). It was seen that the antioxidant nature of the gelatin-curcumin particles was stronger when compared to commercial curcumin powder. The antioxidant property of curcumin is also affected by its extraction method, and it is seen that high heat and pressure extraction proves to be an effective method of extraction and yields improved antioxidant properties, with concentration of ethanol used for extraction being the most important parameter (Choi et al., 2020). Gelatin-chitosan electrospun films were loaded with different concentrations of curcumin to see its effect on different functional and structural properties (Duan et al., 2023). With the addition of curcumin, the films had better thermal stability and tensile strength, as well as improved antioxidant and antimicrobial activities. It proved that the use of curcumin in nanofiber films is of significance and food packaging incorporating curcumin can be made for various foods, such as meat and seafood. Curcumin was also used in intelligent packaging, where it was incorporated into chitosan/PEO and electrospun to produce nanofibers. These were used to indicate colour change when applied to chicken breast package at 4°C, and showed promising results to visualize real-time monitoring of chicken spoilage (Yildiz et al., 2021). The use of curcumin as an antioxidant and anti-microbial agent is further confirmed by a Rho and Rhim's study, where curcumin was added to gelatin solution emulsified with sodium dodecyl sulfate (SDS) and cast to form active films (Roy & Rhim, 2020). Compared to neat gelatin films, the films loaded with curcumin showed incredible activity against pathogens (*E. coli* and *L. Monocytogenes*), as well as antioxidant capacity similar to

that of ascorbic acid. However, the effect of citric acid crosslinking on curcumin functionality in films has not yet been studied .

1.5 Crosslinking

Crosslinking is a process of joining two or more polymer chains together to obtain a polymer with enhanced properties such as lower solubility and higher thermal stability. Crosslinking agents are molecules that have two or more reactive ends and thus have the ability to chemically join to two or more functional groups at a time, such as primary amines, sulfhydryl, and others. One of the commonly used chemical crosslinker for nanofibers is glutaraldehyde, which is now being replaced with other options such as citric acid.

1.5.1 Glutaraldehyde crosslinking

Glutaraldehyde, $\text{CH}_2(\text{CH}_2\text{CHO})_2$ (GA), when reacted with proteins, forms Schiff bases - a kind of double bond linking carbon and nitrogen atoms - between its two carbonyl ends and positively charged amino groups on the protein surface (Le Salem et al., 2010). In a study conducted on electrospun mats from sodium alginate (NaAlg) and poly (vinyl alcohol) (PVA), the mixture was crosslinked with different concentrations (1.25, 2.5 and 5 v %) of glutaraldehyde as well as application time (10 min, 60 min and 24 h) and its effect on the mechanical properties of the resulting products was observed (PAKOLPAKÇIL, 2022). It was seen that with the highest concentration of glutaraldehyde as well as longest application time showed to have the highest tensile strength, thus proving glutaraldehyde to be an effective agent for crosslinking. In another study, dermal sheep collagen (DSC) was crosslinked with glutaraldehyde and its stability was studied (Olde Damink et al., 1995). As a result of this study, an increase in shrinkage temperature was seen in the GA-crosslinked DSC. Although many studies are present that use glutaraldehyde to strengthen the materials being studied, it has recently been found that glutaraldehyde is a toxic

compound to human health, hence it is unadvisable to use it for food packaging. When rats and mice were tested upon regarding the toxicity effect of glutaraldehyde, moderate acute peroral toxicity was found for solutions of 5% and above, and mice were more susceptible than rats in this regard (Ballantyne, 2001). Necrosis, skin irritation and eye irritation also occurred in the tested animals. In a study conducted to see if PVA films could be reinforced with multiwalled carbon nanotubes (MWCNTs) instead of glutaraldehyde, MWCNTs enforced films showed better mechanical properties such as tensile strength and showed antibacterial activity (Mohammad et al., 2013). Thus, it was concluded that these could be used to avoid the residual toxic effects of crosslinking with glutaraldehyde. In a separate study, amide crosslinking was used instead of glutaraldehyde treatment, where tissues of the heart valve were fixed by amine and carboxyl moieties using coupling agents. The by-products were seen to be non-toxic, unlike glutaraldehyde. Thus, it can be said that there are ways to replace the use of glutaraldehyde for use in different industries, namely food, medicine etc.

1.5.2 Citric acid crosslinking

Among the many alternates to glutaraldehyde, including tannic acid or ferulic acid, citric acid is one viable option as well. Citric acid is the most cost-effective choice due to its easy availability from citrus fruits and potential compatibility with the polymer solution of study (gelatin/curcumin). When chitosan/gelatin composite films were made with red cabbage pigment and enterocin CHQS, citric acid was used as a crosslinker (Zhang et al., 2023). The crosslinked packaging films showed to have higher thermal stability, water sensitivity and photosensitivity. The citric acid in these films also contributed to a higher antioxidant capacity of the films. In another study, different non-toxic crosslinking agents were used to crosslink electrospun gelatin nanofibers, one of which was citric acid (Ehrmann, 2021). The aim here was to use the films for tissue engineering and biomedicine, and yet again, it proved to contribute valuably to strengthening the fibers and improving morphology. Citric

acid was also used to crosslink films made with chickpea flour, chitosan and active agent curcumin, using the method of casting (Yildiz, Aydogdu Emir, et al., 2022). The WVP was seen to increase by 62.6% in films containing the highest amount of citric acid (1.5% w/v), whereas antioxidant capacity was seen to decrease. Overall, these films proved to be suitable for application on chicken breast for storage. The effect of citric acid was also seen on modified starch/gelatin composite films made by casting (Kumar et al., 2019). Once again, with the addition of citric acid, functional properties such as swelling index and water barrier properties were seen to improve, proving its compatibility with gelatin and starch as well. Citric acid proves to be biocompatible, non-toxic, and inexpensive, and its carboxyl groups form crosslinks with the hydroxyl groups of the polysaccharide through esterification. Similarly, nucleophilic substitution takes place amongst the carboxyl group of the acid and amino group of protein (Uranga et al., 2020). It has been used as a crosslinking agent in films produced with fish gelatin, but extensive research on its use with bovine gelatin is not available. Porcine gelatin was out of consideration due to its ethical/religious concerns, but the major importance of choosing bovine gelatin, apart from easy availability in comparison to fish gelatin, is its higher proline and hydroxyproline; components of collagens and gelatin necessary for gelation. Total proline plus hydroxyproline values range from 156 residues per thousand to 223 residues per thousand, depending on the source of gelatin (piscine, bovine or porcine), with fish (cold water and warm water fish) having the lowest amounts, indicating their reason for being the least stable gelatins (Haug & Draget, 2009). Thus, bovine gelatin proves to have better gelation and hence better physical and rheological properties.

1.6 Objectives and novelty of the study

Electrospinning has gained increasing interest with the rise in research related to nanotechnology. The non-woven mats produced offer a high range of advantages, including porosity that can be tuned according to the requirement of application, high

surface area to volume ratio and wide range of polymers that can be used in the process (Bhardwaj & Kundu, 2010). Gelatin has been successfully used previously with a formic acid solution to produce nanofibers (Ki et al., 2005). It has also been used to encapsulate curcumin to widen the potential uses of curcumin in the food industry (Gómez-Estaca et al., 2017). However, so far, there is no study on production of bovine gelatin based nanofibers containing curcumin by electrospinning. In addition, crosslinking of these nanofibers with citric acid have not been studied before. Gelatin is a type of protein that can be obtained fairly easily and without religious concerns as well, such as being halal or kosher. If gelatin is procured from an animal slaughtered in accordance with Islamic or Jewish principles, it is considered halal or kosher, respectively. Gelatin has a wide use, from being used as gelling agent, to make glazes and for encapsulating functional agents, among others (BATU, 2015), apart from being biodegradable as well. Gelatin from mammalian sources is known for its better gelling qualities and durable film-forming properties (Said & Sarbon, 2022). In comparison to petroleum-based packaging and with a rise in the demand for food safety, gelatin is gaining increasing attention by researchers. Therefore, the major aim of this study was to produce an active packaging film made primarily of gelatin by using electrospinning to extend the shelf-life of foods, leading to sustainable development and preservation of the environment. This active film consisted of encapsulated curcumin, which could serve as an antioxidant and antimicrobial agent. Curcumin is known for its remarkable oxygen scavenging ability, and has shown to increase the AA of the films (Amani et al., 2022). Another aim of the study is to apply crosslinking to reduce the solubility of gelatin. Crosslinking is the process of intermolecular or intramolecular joining of two or more polymers, to make the polymer solution stronger so that films obtained would be less susceptible to the environmental conditions Citric acid was used as the crosslinking agent for this purpose. Citric acid has been used previously to crosslink many hydrogels, including fish gelatin, as it esterifies the hydroxyl groups in the polymer chains, and has proven to be non-toxic and hence safe to use in the food industry (Mali K. K. et al., 2018). However, no studies are present in

literature that combine gelatin as the base polymer, curcumin as the active agent and citric as a chemical crosslinker, to produce active packaging films for food applications, hence this combination is used in this study. The active electrospun nanofibers produced were characterized in terms of morphology, thermal properties, thermogravimetric analysis, antioxidant capacity, total phenolic content, encapsulation efficiency, antimicrobial activity, FTIR and crystallinity.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) along with ethanol was purchased from Merck (Darmstadt, Germany). Gelatin was purchased from Eti Gıda Sanayi ve Ticaret A.Ş., while citric acid and acetic acid were purchased from Sigma-Aldrich.

2.2 Solution Preparation

Gelatin (20%, 25%, 30%, 35%) (w/v) was put into 40% acetic acid solution and mixed at 900 rpm at 45°C with a magnetic stirrer until it became homogenous. The optimum gelatin concentration in terms of uniformity and size of the nanofibers was found to be 30% gelatin, and hence this was chosen. There-after, curcumin was added to ethanol to prepare 0.5% (w/v) curcumin stock solution. The curcumin stock solution (10 mL) was added to the polymer solutions of gelatin (20 mL) and stirred at 1500 rpm for 30 min at 25 °C. The solution with optimum gelatin concentration, according to the uniformity of fibers as well as their diameters, was crosslinked with citric acid by adding 0.5% and 1% (w/v) citric acid and homogenizing at 25°C and 1500 rpm for 10 min.

The nomenclature of the solutions as well as control are given in Table 2.1, followed by solution and film characterization.

Table 2.1 Nomenclature of solutions and their respective films

Solution/Film name	Contents
GL	30% Gelatin (w/v) solution

Table 2.1 (continued)

GLC	30% GL (w/v) solution mixed with 0.5% curcumin (w/v) in ethanol
GLCCA0.5	30% GL (w/v) solution mixed with 0.5% curcumin (w/v) in ethanol and 0.5% CA (w/v)
GLCCA1	30% GL (w/v) solution mixed with 0.5% curcumin (w/v) in ethanol and 1% CA (w/v)

2.3 Solution Properties

2.3.1 Rheological Properties

The rheological behaviors of solutions were measured by using a controlled strain rheometer (Brookfield Ametek Coaxial Cylinder RST rheometer) with a coaxial cylinder measurement geometry. The solution was poured into the cylinder and shear stress values were recorded with respect to shear rates varying from 0.013 s⁻¹ to 300 s⁻¹. The shear stress (τ) versus shear rate ($\dot{\gamma}$) data was fitted well to the Power Law Equation as stated below:

$$\tau = k(\dot{\gamma}^n) \quad (1)$$

where, τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s⁻¹), k is the consistency index (Pa.sn) and n is the flow behavior index.

2.3.2 Color

The color of curcumin solution was measured by colorimeter (Konica Minolta CR-5 Osaka, Japan). Results were reported in terms of Hunter L, a and b values.

2.3.3 Electrical Conductivity

The electrical conductivities of the film forming solutions (FFS) containing curcumin and citric acid along with the control were measured using a WTW inolab Cond 7110 conductometer (City Germany) at 25 °C. Measurements were done twice.

2.4 Electrospinning

Electrospinning was carried out using Nano-Web 103 (Mersin, Tur-key). Each solution was placed in a syringe (5 mL) with a metallic 21-gauge steel needle (11.53 mm inner diameter), placed horizontally on the syringe pump. This was connected to the positively charged electrode that is powered by a direct current (DC) high voltage supplier. The solution was fed through the metal collector, which was connected to the negatively charged end and covered by aluminum foil at a flow rate of 0.6 mL/h. There was 12 cm between the needle tip and the collector, the voltage was maintained at 15 kV and humidity at 35% (using silica gels). Experiments were performed at 25 ± 1 °C.

2.5 Nanofiber film characterization

In order to determine the properties of the nanofibers for comparison, different characterization tests were performed, including SEM imaging, color analysis, antioxidant capacity, total phenolic content, encapsulation efficiency thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray diffraction (XRD), water vapor permeability (WVP), FTIR and antimicrobial analysis, all of which are explained hereunder.

2.5.1 Morphological Analysis

The morphological characteristics of the fibers fabricated were determined using Field Emission Scanning Electron Microscopy (FESEM) (JEOL, Japan). Samples were stuck on metal stubs and then coated with gold palladium (10 nm). About 50 fibers from each sample were randomly selected from SEM images and their diameters were measured by using Image J software (Maryland, USA).

2.5.2 Color

The color of the films was measured by the same method used for the determination of color of solutions, using a colorimeter (Konica Minolta CR-5 Osaka, Japan). Results were reported in terms of Hunter L, a and b values.

2.5.3 Antioxidant Capacity

The antioxidant capacity of the fibers was measured with the DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) method as stated in Luca et al. (2013), with a few modifications. The nanofibers (0.05 g) were dissolved in 5 mL ethanol/water solution (80:20). DPPH[•] radical solution (3.9 mL) with 25 ppm (2.5 mg DPPH[•]/100 mL methanol) was taken and mixed with 100 μ L of methanol. 100 μ L of nanofiber solutions were also mixed with 3.9 mL DPPH radical solution. Absorption of these was measured at 517 nm (A1) by using UV/VIS spectrophotometer (UV 2450, Shimadzu, Columbia, USA). These were then kept in the dark for 2 h to complete the DPPH solution and curcumin reaction. Then the absorptions of samples were measured spectrophotometrically again (A2). Methanol was used as blank. Concentrations (C1 and C2) were found for A1 and A2 using the calibration curve, respectively, and the antioxidant activities (AA) were calculated according to the equation (2) (de Dicastillo et al., 2019):

$$AA \left(mg \frac{DPPH}{g} \text{ dry weight} \right) = \frac{C_1 - C_2}{W_{sample}} \times V \quad (2)$$

where C1 is the concentration of DPPH[·] immediately after the sample (ppm) and DPPH[·] solution are mixed, C2 is the concentration of DPPH[·] 2 hours after mixing (ppm), V is the volume of solution (mL), W is the amount of nanofiber (g). After this, the antioxidant capacity (%AA) of the electro-spun fibers was expressed by equation (3):

$$AA(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

where A_{control} (A1) and A_{sample} (A2) are the absorbance values of the DPPH solution without and with the presence of the sample solutions.

2.5.4 Total Phenolic Content

The total phenolic content of the solutions with curcumin and control solutions were measured by the modified Folin–Ciocalteu method (Luca et al., 2013). Aqueous ethanol solution of 80% was used to dissolve the fibers and 0.5 mL sample of each was mixed with 2.5 mL 0.2 N Folin-Ciocalteu reagent. These solutions were kept in a dark place for 5 min, and thereafter 2 mL of 75 g/L sodium carbonate solution was added. The mixtures were vortexed at each stage. These were then stored in the dark for 1 h, after which their absorptions were measured using a spectrophotometer at 760 nm (UV 2450, Shimadzu, Columbia, USA). A calibration curve was made using multiple gallic acid concentrations and the total phenolic content of solutions was expressed as gallic acid equivalents (GAE) in milligrams per gram dry weight, using these curves (Aydogdu, Yildiz, et al., 2019)

2.5.5 Encapsulation Efficiency

The encapsulation efficiency of curcumin was determined by using the modified method of Gómez-Estaca et al., (2017). The nanofibers of 0.1 g were dissolved in 22

mL of 80% ethanol and centrifuged at 1500 rpm for 20 minutes. Thereafter, their absorbance was measured at 428 nm and the concentration (g/mL) was found against a calibration curve prepared with curcumin in 80% ethanol. The encapsulation efficiency was found using equation (4):

$$\% \text{Encapsulation efficiency} = \frac{\text{curcumin in nanofibers}}{\text{curcumin in solutions}} \times 100 \quad (4)$$

2.5.6 Thermogravimetric Analysis

TGA 2950 (Exstar TG/DTA 6300, RTI Instruments, Inc., Woodland, USA) was used for thermogravimetric analyses. Roughly 5 mg of nanofiber was heated from 25°C to 600°C at a rate of 10°C/min with nitrogen flowing at a rate of 30 mL/min. Analyses were repeated twice (Yildiz et al., 2021).

2.5.7 Differential Scanning Calorimetry

The thermal analysis of the electro-spun nanofibers was performed using a differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, Waltham, MA, USA). Approximately 5 mg of sample was put into a hermetically sealed aluminum pan. As a reference, an empty pan was used. After cooling to -50°C, each pan was heated to 350°C at a rate of 5°C/min. The glass transition temperature, melting temperature, and degradation temperature of each sample were determined using differential scanning calorimetry (DSC) thermograms. The DSC measurements were carried out in duplicates (Yildiz et al., 2021).

2.5.8 X-ray Diffraction

Crystalline properties of the films were analyzed by X-ray diffractometer (Rigaku Ultima-IV, USA) using copper (Cu) irradiation with 30 mA current and 40 kV energy. Samples were scanned at an angular range of 5° and 70° scanning range with

1°/min scanning rate. The crystallinity degree of the samples was determined by using equation (5):

$$\text{Total Crystallinity (TC)} = \frac{I_c}{I_c + I_a} \quad (5)$$

Where, I_c is integrated intensity of crystalline phase and I_a is the integrated intensity of amorphous phase (Yildiz, Ilhan, et al., 2022).

2.5.9 Thickness of film

In order to measure the thickness of the films, a digital micrometer (LYK 5202, Loyka, Ankara, Turkey) was used and measurements were taken at six different locations on the film. An average of these measurements was noted as the thickness of the film. Measurements were repeated twice for each type of film.

2.5.10 Water Vapor Permeability

The Water Vapor Permeability (WVP) of the fibers was determined by the ASTM E96 method. Cups of 40 mm internal diameter were filled with distilled water in order to create an environment of 100% RH for the nanofibers inside the cup. Nanofibers were stretched over the cups. These cups were then placed into pre-equilibrated desiccator cabinets with a 20% RH and allowed to reach steady state conditions. The cups were weighed every hour. Weight loss versus time was plotted and the water vapor transmission rate was determined from its slope divided by the area of nanofibers that are exposed in the cup. WVP will then be calculated using equation (6) (Yildiz et al., 2021):

$$WVP = \frac{WVTR \times \Delta x}{\frac{R_1 - R_2}{100} \times P_{sat}} \quad (6)$$

where WVTR is the water vapor transmission rate ($\text{gm}^{-2}\text{s}^{-1}$), Δx is the thickness of the film (m), R_1 and R_2 are the relative humidity inside and outside the cups,

respectively, and P_{sat} (Pa) is the saturated water vapor pressure at room temperature. Measurements were repeated twice.

2.5.11 Fourier Transform Infrared (FTIR) Analysis

FTIR analyses of electro-spun nanofibers was carried out by using FTIR spectrophotometer (IRAffinity1, Shimadzu, Kyoto, Japan) in attenuated total reflectance (ATR) mode using a diamond ATR crystal. The infrared regions analysis was recorded with 40 scans over the wavenumber range of 500–4000 cm^{-1} (Aydogdu, Yildiz, et al., 2019).

2.5.12 Antimicrobial Analysis

The antimicrobial activity of the films was determined by the agar disk diffusion method against *S. aureus* (ATCC 29213) and *E. coli* (BC1402) strains. The isolates were incubated at 37°C for 24 h in Nutrient agar (NA, Merck, Darmstadt, Germany) to obtain early-stationary phase cells. The turbidity of the cultures was set to 0.5 McFarland (DEN-1 densitometer; Biosan, Riga, Latvia) (108 cfu/mL). 100 μL of the bacterial suspensions was spread on the Mueller-Hinton Agar (Condalab, Madrid, Spain) plate. Then, the films were cut into 1-cm diameter discs and placed on the plates in an appropriate manner. The plates were incubated at 37 °C for 24 h. At the end of the incubation, the diameters of the inhibition zones formed around the disc were measured. The tests were performed in triplicate (Ben Taheur et al., 2016).

2.6 Statistical Analysis

Analysis of variance (ANOVA) was performed to figure out whether there are noticeable differences between formulations or not, with respect to all separate analysis performed on the solutions and nanofibers ($p \leq 0.05$). One way ANOVA was performed and if significant differences were found, Tukey Multiple Comparison

Test was used to compare variables by using MINITAB statistics software (MINITAB for Windows, Ver-sion 19, Minitab Inc., State College, PA, USA).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Solution properties, fiber morphology and color

The electrical conductivity and rheological properties of solutions were performed to determine their relationship with the fiber morphology, specifically the fiber diameter. Certain process parameters as well as ambient conditions were kept constant, such as temperature and RH of the electrospinning environment, voltage applied and the distance between the positive and negative electrodes. The viscosity of a solution helps to determine the nature of the polymer and the interactions between its constituents. The electrical conductivity gives an idea of fiber elongation and hence the thickness of the fibers to be obtained. It is noted that a solution with higher electrical conductivity will produce thinner fibers, even spider-net fibers, whereas a solution with lower conductivity will produce thicker fibers (Erdem & Akalm, 2015). As the electrostatic interactions of a solution increase, its jet elongates more under the effect of an electric field and hence forms thinner fibers. The electrical conductivity depends on different parameters, such as polymer type, solvent type, concentration of polymer and temperature (Al-Hassan & Norziah, 2012). In terms of the viscosity of the solutions, if a solution is highly viscous, it will not be able to eject from the spinneret and if it has low viscosity, fibers will not be formed (Bhardwaj & Kundu, 2010). Therefore, it was important to note the viscosity and its effect on ease of spinning and fiber morphology. Table 3.1 shows the values of flow behaviour index (n), consistency coefficient (k), average viscosity, electrical conductivity as well as average fiber diameters, obtained from the different solutions and their respective nanofibers.

Table 3.1 Solution properties of polymer solutions and their respective fibre diameters

Solution/Film	n	K (Pa.s.n)	Average viscosity (Pa.s)	Electrical Conductivity (mS/cm)	Fiber diameter (nm)
GL	0.9880 ± 0.0060 ^a	0.441 ± 0.0220 ^a	0.41433 ± 0.0083 ^a	2.74533 ± 0.0019 ^a	162.420 ± 0.916 ^d
GLC	0.9532 ± 0.0243 ^a	0.2458 ± 0.0060 ^b	0.16786 ± 0.0117 ^b	1.30757 ± 0.0032 ^b	182.246 ± 0.619 ^c
GLCCA0.5	0.9688 ± 0.0031 ^a	0.1749 ± 0.0087 ^b	0.14931 ± 0.0102 ^b	1.29360 ± 0.0048 ^b	344.033 ± 0.304 ^b
GLCCA1	0.9684 ± 0.0175 ^a	0.1654 ± 0.0341 ^b	0.14040 ± 0.0165 ^b	1.17300 ± 0.0093 ^c	427.417 ± 1.390 ^a

*Columns with different letters are significantly different to each other (p < 0.05).

As can be seen in Table 3.1, the viscosity of solutions decreased as curcumin and citric acid are added to the samples. On the other hand, their electrical conductivity decreased. A Newtonian characteristic can be seen as the n values were close to 1. The significant decrease in the viscosity value in the presence of curcumin addition was majorly due to addition of ethanol decreasing the viscosity of the gelatin solution, i.e., diluting it. This was a pattern that was seen in previous studies as well. When caffeic acid was dissolved in an ethanol-water solution and then added to carob bean-WPC-PEO solution, the solution was seen to become less viscous (Zeren et al., 2022). Similarly, upon addition of gallic acid to lentil flour-based nanofiber solutions, it was observed that the consistency index (k) values decreased, which was an indication of decreasing viscosity (Aydogdu, Sumnu, et al., 2019). Furthermore, viscosity was also seen to increase with increasing gelatin concentration from 7 to 20% (w/v) (Al-Hassan & Norziah, 2012). From this, it can be inferred that the decreasing viscosity in our solution was expected as the overall concentration of gelatin in the solutions decreased when curcumin mixed with ethanol was added, and further upon addition of citric acid.

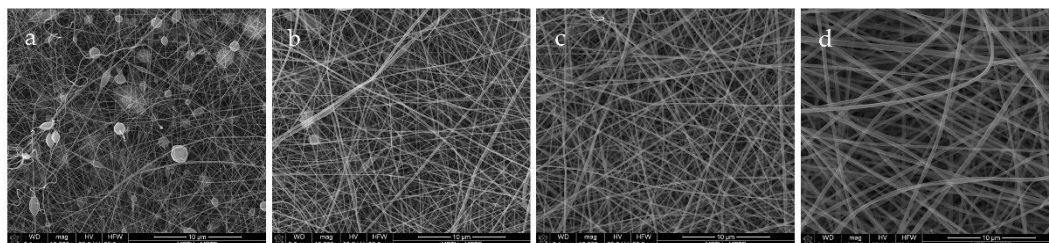


Figure 3.1 Nanofiber morphology for different gelatin concentrations (a) 20%GL, (b) 25%GL, (c) 30%GL, (d) 35%GL

Figure 3.1 shows the fiber morphologies through SEM images of fibers with different gelatin concentrations, varying from 20%-35%. This experiment was performed to determine the optimum gelatin concentration by considering the size and uniformity of fibers. As can be seen, the 20% and 25% GL nanofibers were non-uniform and contained beads, both of which were unacceptable attributes in electro-spun fibers. Hence, due to the uniformity and neatness of 30% and 35% GL, the study was narrowed down to these concentrations. Thus, curcumin solution was

added to both and based on the nanofibers with a lower diameter (182.246 nm for 30% GLC and 263.754 nm for 35%GLC), the 30% GL with curcumin stock solution added was chosen before adding citric acid.

Figure 3.2 shows the morphology of all four different fiber types (GL, GLC, GLCCA0.5, GLCCA1). The diameters increased from GL to GLCCA1 (161.8 nm to 426.4 nm). It is known that the diameters of electro-spun fibers are highly dependent on the electrical conductivities of their respective solutions, with a higher electrical conductivity leading to smaller fiber diameters, a case seen in this study as well (Table 3.1). This is due to higher electrostatic forces and hence longer jet elongation under the voltage applied in the electrospinning machine, leading to thinner diameters. Surface tangential stress is created when charge is more easily accumulated in a solution due to higher conductivity, which aids in fiber elongation (Williams et al., 2021). Solutions with zero electrical conductivity cannot produce fibers, whereas if the conductivity is too high, electrical discharge into the surrounding occurs. It was seen that when the conductivity of PEO solution was increased to 0.5 S m^{-1} (5 mScm^{-1}), the stable cone jet changed to a multi-jet expulsion from the needle (Morota et al., 2004) and the solution could not be spun. Previously, gelatin was doped with formic acid by dissolving gelatin solution in formic acid solution to obtain improved properties, such as enhanced stability, in the nanofibers (Ki et al., 2005). The fiber diameters increased upon addition of formic acid, and this was parallel to the diameters increasing with citric acid addition for crosslinking.

Table 3.2 shows the L, a and b values of the solution and their corresponding films, measured according to the Hunter Lab scale. Focus was drawn towards the lightness (L) and yellowness (b) of the solution and films. The solution became lighter upon the addition of citric acid, in comparison to solutions with only gelatin and curcumin (GLC), owing to the pH sensitivity of curcumin and protein structure of gelatin. A drastic value change was seen in the b values (blue-yellow scale), where both the solution and films became more yellow upon addition of curcumin, an expected change due to the rich yellow pigment of curcumin.

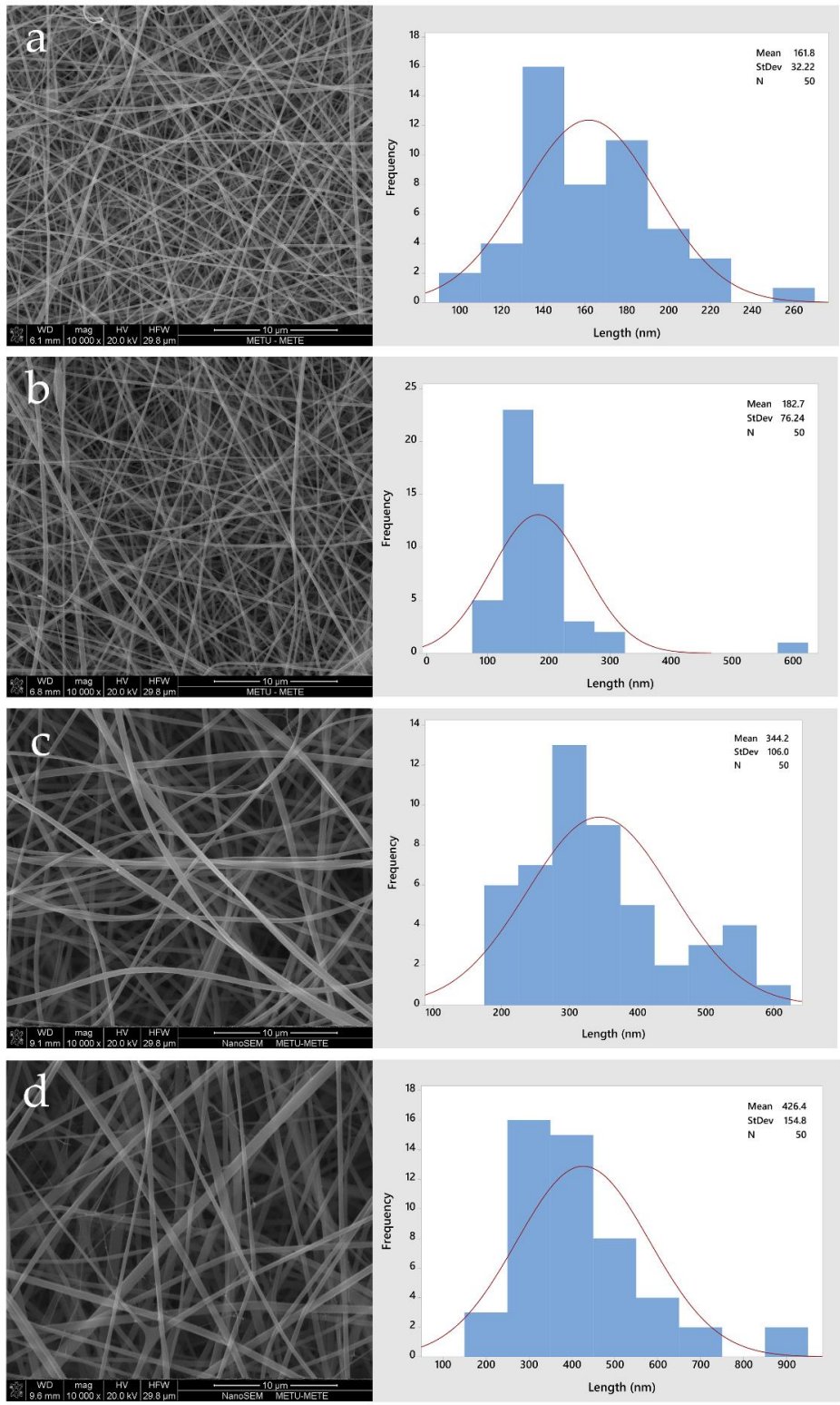


Figure 3.2 SEM images and fiber size distributions: (a) GL (b) GLC (c) GLCCA0.5 (d) GLCCA1

Table 3.2. Color results (L*, a* and b* values) of the solution and the films

Type	L	a	b	
GL	Solution	90.6350 ± 0.0071 ^a	-3.980 ± 0.2260 ^a	17.330 ± 0.325 ^c
	Film	19.255 ± 0.2900 ^b	-1.190 ± 0.141 ^c	1.200 ± 0.226 ^c
GLC	Solution	82.0350 ± 0.0778 ^c	4.350 ± 0.0283 ^c	56.865 ± 0.0495 ^b
	Film	13.915 ± 0.2620 ^c	5.330 ± 0.382 ^a	8.345 ± 0.304 ^b
GLCCA0.5	Solution	83.120 ± 0.1980 ^b	2.430 ± 0.0283 ^b	57.790 ± 0.156 ^{ab}
	Film	20.605 ± 0.4030 ^a	3.350 ± 0.382 ^b	13.170 ± 0.339 ^a
GLCCA1	Solution	83.4800 ± 0.1273 ^b	2.205 ± 0.191 ^b	57.990 ± 0.311 ^a
	Film	14.540 ± 0.0424 ^c	3.845 ± 0.191 ^b	9.325 ± 0.460 ^b

*Columns with different letters are significantly different to each other (p < 0.05).

3.2 Antioxidant capacity

Curcumin is known to scavenge free radicals and hence its antioxidant capacity was determined. It is known that the antioxidant capacity of curcumin originates from its phenolic hydroxyl group and ethylene group of β -diketone moiety (Priyadarsini et al., 2003). As can be seen in Table 3.3, it was found that the antioxidant capacity of the nanofiber films decreased significantly upon addition of citric acid for crosslinking, and thereafter increased significantly as well, upon addition of a higher percentage of citric acid. It is also known that the carboxyl groups of citric acid react with the active sites of curcumin (Yildiz et al., 2021), a reaction that may lead to a reduction in the number of active sites available for the antioxidant capacity of curcumin. This decrease in AA is also attributed to crosslinking, as the release rate is expected to be lower in a crosslinked polymer matrix and hence its resulting films have relatively lower antioxidant capacity (Valizadeh et al., 2019). Contrary to this finding, ineffective encapsulation of curcumin and crosslinking in the film can also be inferred from the fact that the antioxidant capacity of the films (due to curcumin) has not increased in the presence of citric acid (Yang et al., 2010), and, in fact, decreased by almost 30%. However, upon addition of a higher amount of the crosslinking agent, a slight increase in AA was seen from GLCCA0.5 to GLCCA1, by another 24%. This could be attributed to the fact that due to the low amount of curcumin, the additional citric acid might not have bound to its active sites, and the AA might have resulted from the additional citric acid, not from the curcumin itself (Priyadarshi et al., 2018).

Table 3.3 Antioxidant capacity of films

Film	AA (%)
GLC	32.01 \pm 1.88 ^a
GLCCA0.5	22.47 \pm 0.63 ^b
GLCCA1	27.93 \pm 0.78 ^a

*Columns with different letters are significantly different to each other ($p < 0.05$).

AA: Antioxidant capacity.

3.3 Total Phenolic Content and Encapsulation Efficiency

The Total Phenolic Content (TPC) is an important measure as curcumins and all curcuminoids are known to be rich in phenols and polyphenols, which contribute to the antioxidant capacity of spices and herbs (Shan et al., 2005; Wong et al., 2006). It was also important in giving an understanding of the encapsulation efficiency of the solution, i.e., how effectively curcumin was incorporated into the solution. It is shown in Table 3.4 that the TPC of GLC (0.35495 mg GAE/g) was higher than that of GLCCA0.5 (0.3228 mg GAE/g). Other studies reported various TPCs of curcumin and curcumin related items; curcumin extract 0.6632 mg GAE/g (Khalid Saeed et al., n.d.) pure curcumin 11.24 mg GAE/g (Himesh et al., 2011), fresh turmeric 13.4 mg GAE/g (Tangkanakul et al., 2009) and dried turmeric 0.0172-0.0746 mg GAE/g (Wojdyło et al., 2007). For GLCCA1, the TPC decreased significantly in comparison to GLC, indicating lower encapsulation in the presence of citric acid. This is confirmed by looking at the encapsulation efficiency (EE) data, where all films appear to have an encapsulation efficiency between 45% and 60% and it is consistent with the order of highest to lowest TPCs of the films (highest for GLC and lowest for GLCCA1). Generally, the EE of curcumin in proteins is known to be near to 90% or above, as indicated by its encapsulation in whey protein, zein and β -lactoglobulin (Solghi et al., 2020). It is also stated that curcumin in gelatin had an EE of $97 \pm 5\%$ when electro-sprayed (Gómez-Estaca et al., 2017). In this study, pig skin gelatin was used with a bloom value of 300g, whereas the gelatin used in our study was bovine gelatin with a 200g bloom value. The amount of curcumin used was also about 12 times more, leading to better loading into the solution. This may be a reason for lower encapsulation of curcumin in our study than in the study of Gomez-Estaca et al (2015) (Gómez-Estaca et al., 2015). In another study by Alehosseini et al., the encapsulation efficiency of curcumin in 20% GL (w/w) was 89%, where curcumin was added into the gelatin solution after being dissolved in an ethanolic phosphatidylcholine solution and forming liposome dispersions in acetic acid, so that its poor solubility in aqueous solutions could be overcome (2019). From this it can

be inferred that the encapsulation of sensitive bioactive compounds such as curcumin may depend on the types of solvents used and technique used (electrospinning/electrospraying). Owing to the fact that only ethanol was used to dissolve curcumin and the percentage of acetic acid was also different in the primary solution (40%), the encapsulation efficiency of the resulting electrospun nanofibers was lower. In other words, since gelatin was dissolved in a 40% acetic acid solution, a major part (60%) of this solution was aqueous, leading to the curcumin solution being ineffectively encapsulated into it. Other than that, no significant difference can be seen between the EE of GLC and GLCCA0.5, but a significant difference was seen between GLC and GLCCA1, indicating that the quantity of citric acid has an effective role in impacting the encapsulation of curcumin in the fibres.

Table 3.4 Total Phenolic content and Encapsulation Efficiency of films

Film	TPC mg GAE/g	EE %
GLC	0.35495 ± 0.0070 ^a	60.696 ± 1.197 ^a
GLCCA0.5	0.3228 ± 0.0175 ^{ab}	55.19 ± 2.990 ^{ab}
GLCCA1	0.2634 ± 0.0175 ^b	45.04 ± 2.990 ^b

*Columns with different letters are significantly different to each other ($p < 0.05$).

TPC: Total Phenolic Content, EE: Encapsulation Efficiency.

3.4 Thermogravimetric analysis

The thermogravimetric analysis was performed to understand the weight loss variation of the films with increasing temperature. TGA gives an idea of certain thermal events that occur on the films, such as absorption, adsorption, vaporization, decomposition, etc. (Loganathan et al., 2017). Figure 3.3 shows the weight loss versus temperature graphs of the nanofiber films, along with Table 3.5 showing the weight loss percentages. It can be noted that the films follow a two-step weight loss, with the first one being responsible for evaporation of water and other volatile compounds, centered around 100-150°C (Yildiz, Ilhan, et al., 2022). The second stage weight loss is due to evaporation of citric acid derivatives in the film (Qiao et

al., 2021), followed by decomposition linked to depolymerization reactions (Wu et al., 2019) and covalent bond cleavage (Sharma et al., 2018). The peak of the second stage is centered around 400°C. Upon addition of curcumin and further upon addition of the crosslinking agent, citric acid, the weight losses are seen to decrease slightly, indicating the occurrence of crosslinking and strong intermolecular interactions between the components of the polymer solution.

Table 3.5 Two stage weight loss percentage and crystallinity percentage of films

Film	Weight loss 1 (%)	Weight loss 2 (%)	Crystallinity (%)
GL	11.110 ± 0.4330 ^a	61.430 ± 0.2520 ^b	2.220 ± 0.2520 ^b
GLC	10.200 ± 1.7140 ^{ab}	60.960 ± 0.1153 ^b	1.626 ± 0.3150 ^b
GLCCA0.5	8.763 ± 0.9120 ^{ab}	60.907 ± 0.5550 ^b	6.50 ± 1.5400 ^a
GLCCA1	8.047 ± 0.5530 ^b	63.180 ± 0.9430 ^a	1.5625 ± 0.0898 ^b

*Columns with different letters are significantly different to each other (p < 0.05).

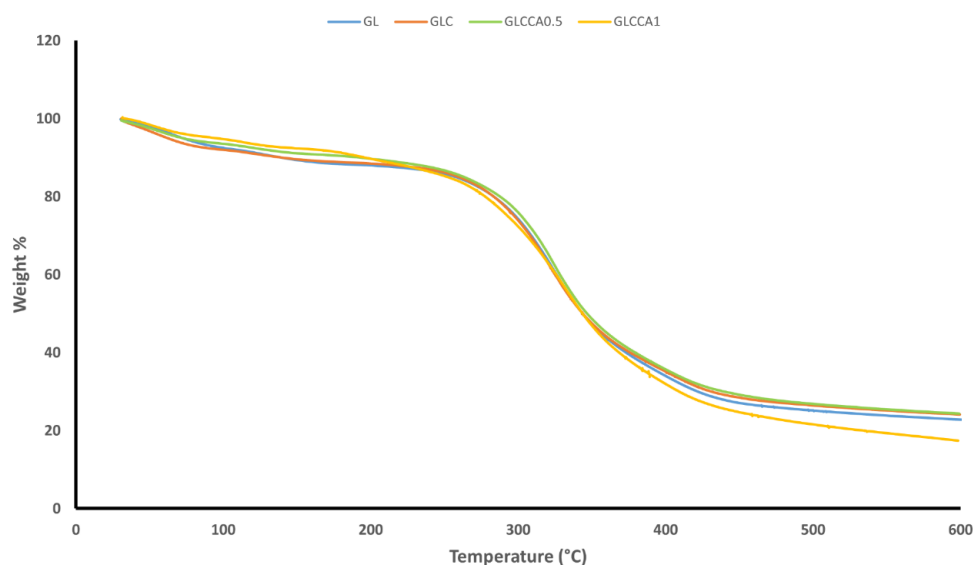


Figure 3.3 TGA weight loss graphs

3.5 Differential Scanning Calorimetry

This thermo-analytical technique provided information on the difference in energy and heat required to increase the temperature in the samples. The glass transition temperature, T_g , shown by a shift in the graph was seen between 68°C and 120°C, related to the glass-to-rubber transition of amino acid blocks in the peptide chain (Rawdkuen et al., 2010). This was followed by the melting temperature, T_m , indicated by an endothermic peak, between 70°C and 80°C. T_g is the softening point of the films and T_m represents the dissociation of the ordered regions or melting point of the crystal phase of the material. Table 3.6 shows the T_g , T_m (along with onset and end temperatures) as well as degradation temperatures of the films. The films show a semi-crystalline structure due to presence of both T_g and T_m , and the fibers mostly remain in non-crystalline state as a result of elongating and rapidly solidifying during electrospinning (Yildiz, Ilhan, et al., 2022). GL films melt at a similar temperature to that studied in bovine gelatin films earlier (Rawdkuen et al., 2010) but show a higher T_g than gelatin films produced through casting (Suderman et al., 2018). An increase in T_g is seen upon addition of curcumin in gelatin and this may be due to the curcumin causing inflexibility in the amorphous polymer chain movement, higher hydrogen bonding and stronger crystalline forces (Jadhav et al., 2009). The films were also seen to become less sticky, which was an expected observation upon increase in T_g . Further upon addition of citric acid, the T_g was seen to decrease in comparison to GLC. Since this T_g was greater than the T_g for GL, it can be an indication of crosslinking (Jadhav et al., 2009), although there is also an indication of an increase in the free volume between the polymer chains. An overall decrease in melting temperature was seen as curcumin and citric acid were added, associated with the strengthening of the polymer solution with hydrogen bonding. By looking at the degradation temperature data, an increase in the amorphous nature of the polymer solution due to crosslinking was noticed in gelatin fibers prepared from gelatin-formic acid solution, wherein there was a higher amount of random coil conformation and lower helical conformation (Ki et al., 2005). Even

though an overall decrease in degradation temperature was also seen upon addition of the cross-linking agent in the films in this study, this decrease was not significant and therefore a noteworthy change in conformation could not be concluded.

Table 3.6 Glass transition (Tg), Melting (Tm) and Degradation Temperatures (Tdeg) of films

Film	Tg (°C)	Tm start (°C)	Tm peak (°C)	Tm end (°C)	T deg (°C)
GL	69.16 ± 1.460 ^c	55.10 ± 1.57 ^a	80.285 ± 0.728 ^a	106.36 ± 4.40 ^b	231.515 ± 1.068 ^a
GLC	119.730 ± 1.117 ^a	48.98 ± 2.93 ^a	73.835 ± 1.308 ^c	98.52 ± 8.79 ^b	227.745 ± 1.280 ^a
GLCCA0.5	112.73 ± 1.640 ^b	32.35 ± 2.80 ^b	78.055 ± 1.407 ^{ab}	142.69 ± 3.20 ^a	230.140 ± 2.570 ^a
GLCCA1	110.49 ± 1.440 ^b	46.56 ± 2.70 ^a	76.010 ± 0.170 ^{bc}	103.25 ± 3.14 ^b	226.050 ± 1.004 ^a

*Columns with different letters are significantly different to each other (p < 0.05). Tg: glass transition temperature; Tm: melting temperature.

3.6 Crystallinity Analysis (XRD)

To understand the change in crystallinity of the gelatin films with respect to curcumin solution and citric acid, the XRD analysis was performed, and its resulting graphs are shown in Figure 3.4. As shown in Figure 3.4 and Table 3.5, the crystallinity was generally quite low for the films and an overall decreasing trend could be seen. In a previous study (Ki et al., 2005), it was stated that the crystalline structure of gelatin results from its α -helix and triple helical structure and that the crystallinity of aqueous gelatin solutions is lower, with more amorphous structures seen in nanofibers (based on a gelatin and formic acid mixture). Similarly, crystallinity of GL was decreasing in GLC and further in GLCCA because the carboxyl groups of CA might be reacting with the hydroxyl and amine groups of the protein, causing hindrance in the formation of crystal structure (Yildiz, Ilhan, et al., 2022). The crystallinity is also hindered due to the NH₂ groups in gelatin favoring hydrogen bond formation in the polymer chain, further strengthening the film (Pavoni et al., 2021).

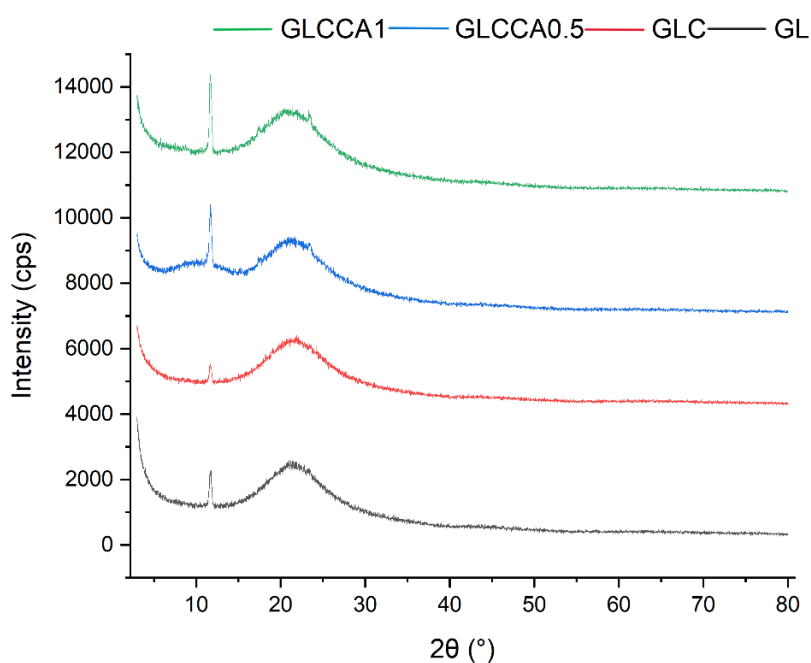


Figure 3.4 XRD graphs of films

3.7 Water Vapor Permeability

The main function of testing water vapor permeability is to determine how strong the water barrier in a packaging film is, between the food product being packaged and the surrounding. Water vapor permeability (WVP) of the films is aimed to be as low as possible. It is generally seen for hydrophilic films, such as gelatin, that the WVP increases with increasing thickness of films. Even though the films in this study were very thin (~0.05-0.08 mm), the nanofibers provided a good barrier for water and there was no significant difference seen in WVP. Overall, it can be said that the WVP was quite low, as shown in Table 3.7. In a study conducted to compare the WVP of fish gelatin with that of mammalian gelatin (bovine and porcine), mammalian gelatin was found to have a higher WVP due to higher amounts of proline and hydroxyproline in its structure, causing it to have a higher affinity for moisture (Avena-Bustillos et al., 2006). In it, the WVP of gelatin films was higher than that seen in Table 3.7 by about 20%, which was attributed to the solvent used (water) for dissolution as well as casting method used instead of electrospinning. Similar to our results, starch-fish gelatin films also did not show a significant difference in their WVP when the starch-gelatin ratios were changed and plasticized with glycerol (Al-Hassan & Norziah, 2012). WVP depends on many factors, including the ratio between crystalline and amorphous zones, interactions between functional groups etc. (García et al., 2000), but the slight difference seen could be due to difference in water molecule diffusion (through the nanofiber matrix), hydrophilic-hydrophobic ratio (Cano Embuena et al., 2015) and viscosity of film forming solutions. The drop from 4.395×10^{-10} to 3.590×10^{-10} could be linked to the hydrophobic nature of curcumin, which might have a decreasing effect on water solubility (Roy & Rhim, 2020). Upon addition of citric acid, the WVP was seen to be higher than that of faba bean-chitosan-curcumin films crosslinked with citric acid, since citric acid was known to be better at forming hydrophobic ester bonds with polysaccharides than with proteins (Yildiz, Ilhan, et al., 2022).

Table 3.7 Water Vapor Permeability and thickness of films

Film	WVP ($\times 10^{-10}$) $\text{gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$ unit	Thickness (mm)
GL	4.395 ± 0.000^a	0.088 ± 0.0035^a
GLC	3.590 ± 0.000^{bc}	0.059 ± 0.0081^b
GLCCA0.5	2.365 ± 0.000^c	0.050 ± 0.0034^b
GLCCA1	4.695 ± 0.000^{ab}	0.082 ± 0.0027^a

*Columns with different letters are significantly different to each other ($p < 0.05$).

3.8 Fourier Transform Infrared Analysis (FTIR)

The FTIR analysis was conducted in order to identify characteristic peaks and functional groups present in the thin films. The characteristic amide peaks (Amide I, Amide II, Amide III and Amide A) peaks of gelatin were seen in all four films with slight differences in wavenumbers, as shown in Figure 3.5. The peak at 1640 cm^{-1} (Amide I) corresponds to the C=O stretching vibration of gelatin, linked to the random coil and α -helix conformation of gelatin (Prystupa & Donald, 1996) (Muyonga et al., 2004). At 1530 cm^{-1} (Amide II) and 1240 cm^{-1} (Amide A) the peaks correspond to bending of N-H and stretching of C-N, respectively (Prystupa & Donald, 1996), while N-H stretching is seen between $3100\text{-}3500 \text{ cm}^{-1}$, with a peak at 3290 cm^{-1} . No functional groups are altered upon addition of substances to the polymer solution, verified by the fact that all characteristic peaks of GL are present in the rest as of the films as well (Kotatha et al., 2019). However, as a change in intensity of the peaks can be seen, with the largest difference seen in GLCCA1 for all peaks, it can be concluded that the helix or protein secondary structure has been altered (Rawdkuen et al., 2010) and therefore some crosslinking has occurred.

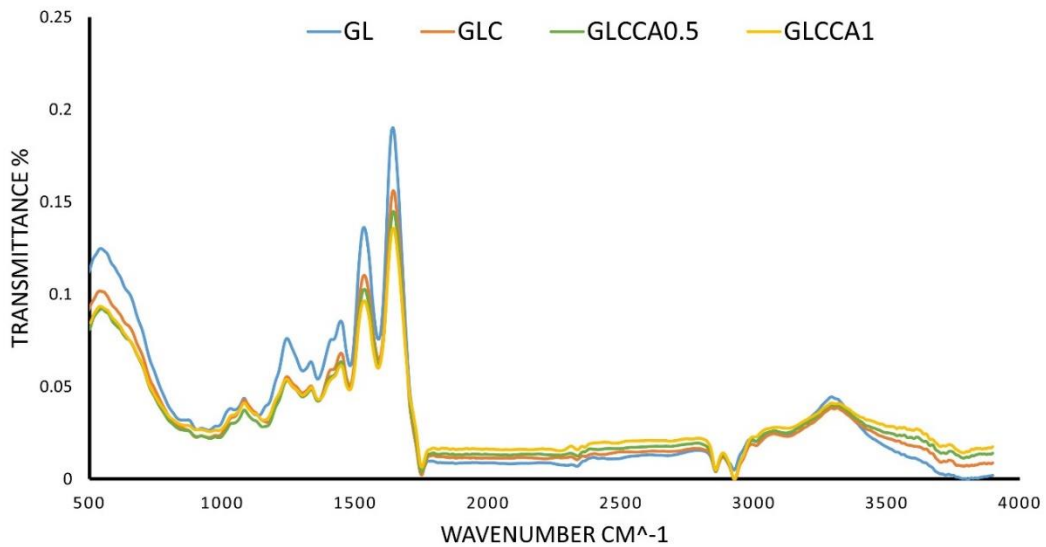


Figure 3.5 FTIR spectra of films

3.9 Antimicrobial Activity

Antimicrobial tests were conducted on the films and the presence of inhibition zones were investigated, against *E. coli* and *S. aureus*, as shown in Figure 3.6. Even though these films showed significant antioxidant capacity, no inhibition zones were seen on all the films. Similarly, it was determined that although the gelatin/curcumin-based films had significant antioxidant properties, they did not exhibit antimicrobial activity against *E. coli* and *S. aureus* (Musso et al., 2017). Since the antimicrobial activity was largely dependent on curcumin with GL being used as the control film, the main reason for no inhibition in the present study could be the low concentration of curcumin. Similar results were obtained previously when sumac was incorporated into faba bean flour active films (Emir et al., 2023). In it, there was no antimicrobial activity detected with 5% sumac, for *S. aureus*, but it increased significantly when the sumac amount was increased to 10% and 20%. In this regard, it has been reported that the minimum concentration required for aqueous extraction of *C. longa* to show antimicrobial activity against *E. coli* and *S. aureus* was 4-32 g/L (Niamsa & Sittiwet, 2004). In another study, it was stated that the minimum inhibitory concentration

value of ethanolic turmeric extract with *S. aureus* was 31.25 ppt (Lawhavinit et al., 2010). In addition, one of the causes of curcumin's antimicrobial activity is its ability to alter membrane permeability and inhibit bacterial growth (Tyagi et al., 2015). Hydrogen bonding and hydrophobic interactions of bacterial cell phenols and membrane proteins are responsible for this effect of curcumin on bacteria. However, in protein-based films, proteins can interact with phenolic compounds such as curcumin, resulting in blocking the active sites desired for antimicrobial activity (Aliabbasi et al., 2021). It was found earlier that protein films loaded with phenolic compounds did not provide antimicrobial activity, and it was reported that this result may be related to the interactions of phenolic compounds with proteins and the alkaline pH of the film dispersions (Salgado et al., 2012). Apart from these, it was found that nanocurcumin had a higher inhibition zone than bulk curcumin and was a more effective antimicrobial agent due to the reduced particle size (Hettiarachchi et al., 2022). In light of the literature data, it is thought that the lack of antimicrobial activity of the films obtained in the present study may be due to the nature of curcumin, the low concentration of curcumin, and possible interactions between gelatin and curcumin.

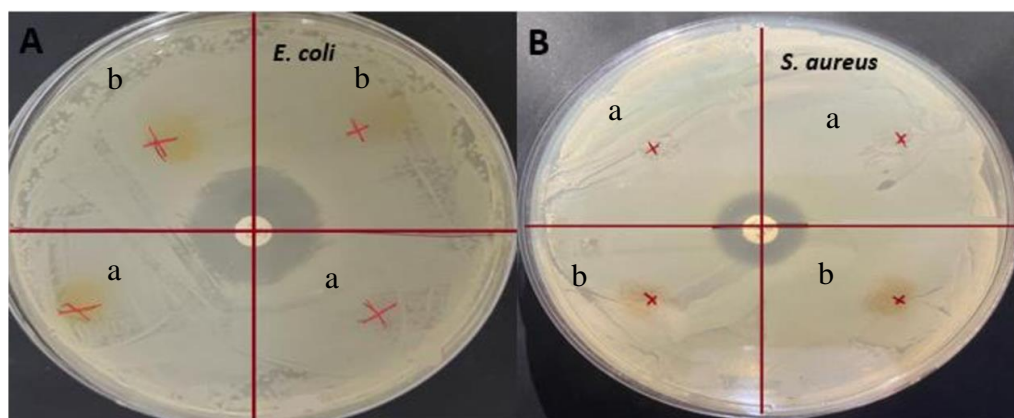


Figure 3.6 Antimicrobial analysis (a) GLCCA0.5 (b) GLCCA1

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

This study was aimed at employing the method of electrospinning with bovine gelatin as the base, curcumin added as functional agent and citric acid used for crosslinking, to produce a packaging film with enhanced antioxidant activities. The viscosity of the solutions decreased almost threefold as curcumin and citric acid were added, in comparison to gelatin solutions, and the electrical conductivity was halved. It was seen upon addition of curcumin and further upon addition of citric acid, that the diameter of the fibers increased significantly, but uniform fibers were still obtained. As electrical conductivity decreases, fiber diameter is known to increase, and this pattern was seen in this study as well. The antioxidant capacity of GLC was seen to be the highest, and its weight loss and crystallinity data was seen to be promising for it to be used as a packaging film. In GLCCA0.5 and GLCCA1, a few properties, such as antioxidant capacity, thermal properties and WVP, were enhanced while others marred or unchanged, such as film T_{deg} . Between 0.5% and 1% citric acid addition, the 1% citric acid crosslinked films were the winning candidates with respect to improved amorphous nature (decreased crystallinity) and antioxidant capacity. On the other hand, GLCCA0.5 were better at being thermally stable and provided the strongest barrier against water. Therefore, curcumin and citric acid paired with gelatin are a promising combination to be used as a packaging material for cut fruits.

As a further study, it is recommended to use a higher concentration of citric acid (about 5-10%) for better crosslinking capability, or to replace the crosslinking agent with another, such as ferulic acid, to see whether it proves to be better at crosslinking gelatin. Heating the solution once the crosslinking agent is added may be helpful in forming covalent bonds required to strengthen the films, and using a higher concentration of curcumin in ethanol may also help with better loading and

encapsulation. Gelatin of higher strength may be used as it can provide a chance for stronger films, and this gelatin may be dissolved in glacial acetic acid instead of 40% acetic acid so that the curcumin can be better incorporated into the solution.

REFERENCES

- Alehosseini, A., Gómez-Mascaraque, L. G., Martínez-Sanz, M., & López-Rubio, A. (2019). Electrospun curcumin-loaded protein nanofiber mats as active/bioactive coatings for food packaging applications. *Food Hydrocolloids*, *87*, 758–771. <https://doi.org/10.1016/j.foodhyd.2018.08.056>
- Al-Hassan, A. A., & Norziah, M. H. (2012). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, *26*(1), 108–117. <https://doi.org/10.1016/j.foodhyd.2011.04.015>
- Aliabbasi, N., Fathi, M., & Emam-Djomeh, Z. (2021). Curcumin: A promising bioactive agent for application in food packaging systems. *Journal of Environmental Chemical Engineering*, *9*(4). <https://doi.org/10.1016/j.jece.2021.105520>
- Alves, J., Gaspar, P. D., Lima, T. M., & Silva, P. D. (2022). *What is the role of active packaging in the future of food sustainability? A systematic review*. <https://doi.org/10.1002/jsfa.11880>
- Amani, F., Rezaei, A., Akbari, H., Dima, C., & Jafari, S. M. (2022). Active Packaging Films Made by Complex Coacervation of Tragacanth Gum and Gelatin Loaded with Curcumin; Characterization and Antioxidant capacity. *Foods*, *11*(20). <https://doi.org/10.3390/foods11203168>
- Avena-Bustillos, R. J., Olsen, C. W., Olson, D. A., Chiou, B., Yee, E., Bechtel, P. J., & McHugh, T. H. (2006). Water vapor permeability of mammalian and fish gelatin films. *Journal of Food Science*, *71*(4). <https://doi.org/10.1111/j.1750-3841.2006.00016.x>
- Aydogdu, A., Sumnu, G., & Sahin, S. (2019). Fabrication of gallic acid loaded Hydroxypropyl methylcellulose nanofibers by electrospinning technique as

- active packaging material. *Carbohydrate Polymers*, 208, 241–250. <https://doi.org/10.1016/j.carbpol.2018.12.065>
- Aydogdu, A., Yildiz, E., Aydogdu, Y., Sumnu, G., Sahin, S., & Ayhan, Z. (2019). Enhancing oxidative stability of walnuts by using gallic acid loaded lentil flour based electrospun nanofibers as active packaging material. *Food Hydrocolloids*, 95, 245–255. <https://doi.org/10.1016/j.foodhyd.2019.04.020>
- Ballantyne B, M. R. (2001). The acute toxicity and primary irritancy of glutaraldehyde solutions. *Veterinary and Human Toxicology*, 43(4), 193–202.
- Barbosa-Pereira, L., Aurrekoetxea, G. P., Angulo, I., Paseiro-Losada, P., & Cruz, J. M. (2014). Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef. *Meat Science*, 97(2), 249–254. <https://doi.org/10.1016/J.MEATSCI.2014.02.006>
- BATU, A. (2015). Müslüman Toplumlarında Helal Gıda Üretiminde Jelatin Problemi. *Journal of Turkish Studies*, 10(Volume 10 Issue 14), 37–37. <https://doi.org/10.7827/turkishstudies.8928>
- Ben Taheur, F., Kouidhi, B., Fdhila, K., Elabed, H., Ben Slama, R., Mahdouani, K., Bakhrouf, A., & Chaieb, K. (2016). Anti-bacterial and anti-biofilm activity of probiotic bacteria against oral pathogens. *Microbial Pathogenesis*, 97, 213–220. <https://doi.org/10.1016/j.micpath.2016.06.018>
- Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. In *Biotechnology Advances* (Vol. 28, Issue 3, pp. 325–347). <https://doi.org/10.1016/j.biotechadv.2010.01.004>
- Bojorges, H., Ríos-Corripio, M. A., Hernández-Cázares, A. S., Hidalgo-Contreras, J. V., & Contreras-Oliva, A. (2020). Effect of the application of an edible film with turmeric (*Curcuma longa* L.) on the oxidative stability of meat. *Food Science and Nutrition*, 8(8), 4308–4319. <https://doi.org/10.1002/fsn3.1728>

- Boonpratum, C., Naemchanthara, P., Limsuwan, P., & Naemchanthara, K. (2022). Effects of chitosan and Tween 80 addition on the properties of nanofiber mat through the electrospinning. *E-Polymers*, 22(1), 234–248. <https://doi.org/10.1515/epoly-2022-0029>
- Cai, L., Shi, H., Cao, A., & Jia, J. (2019). Characterization of gelatin/chitosan polymer films integrated with docosahexaenoic acids fabricated by different methods. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-44807-x>
- Camo, J., Beltrán, J. A., & Roncalés, P. (2008). Extension of the display life of lamb with an antioxidant active packaging. *Meat Science*, 80(4), 1086–1091. <https://doi.org/10.1016/J.MEATSCI.2008.04.031>
- Cano Embuena, A. I., González Martínez, C., & Chiralt Boix, A. (2015). *UNIVERSITAT POLITÈCNICA DE VALÈNCIA Different strategies to improve the functionality of biodegradable films based on starch and other polymers DOCTORAL THESIS*.
- Chiellini, E., Cinelli, P., Corti, A., & Kenawy, R. (n.d.). *Composite films based on waste gelatin: thermal-mechanical properties and biodegradation testing*. Retrieved August 1, 2023, from www.elsevier.com/locate/polydegstab
- Choi, Y., Kim, W., Lee, J. S., Youn, S. J., Lee, H., & Baik, M. Y. (2020). Enhanced Antioxidant Capacity of Puffed Turmeric (*Curcuma longa* L.) by High Hydrostatic Pressure Extraction (HHPE) of Bioactive Compounds. *Foods*, 9(11). <https://doi.org/10.3390/foods9111690>
- Dalla, E., Koumentakou, I., Bikiaris, N., Balla, E., Lykidou, S., & Nikolaidis, N. (2022). Formulation, Characterization and Evaluation of Innovative O/W Emulsions Containing Curcumin Derivatives with Enhanced Antioxidant Properties. *Antioxidants*, 11(11). <https://doi.org/10.3390/antiox11112271>
- de Dicastillo, C. L., Piña, C., Garrido, L., Arancibia, C., & Galotto, M. J. (2019). Enhancing thermal stability and bioaccessibility of açai fruit polyphenols

- through electrohydrodynamic encapsulation into zein electrosprayed particles. *Antioxidants*, 8(10). <https://doi.org/10.3390/antiox8100464>
- Deng, L., Kang, X., Liu, Y., Feng, F., & Zhang, H. (2017). *Characterization of gelatin/zein films fabricated by electrospinning vs solvent casting*. <https://doi.org/10.1016/j.foodhyd.2017.08.023>
- Dong, Y., Rao, Z., Liu, Y., Zheng, X., Tang, K., & Liu, J. (2023). *Soluble soybean polysaccharide/gelatin active edible films incorporated with curcumin for oil packaging*. <https://doi.org/10.1016/j.fpsl.2023.101039>
- Du, J., & Zhang, X. (2008). Role of polymer–salt–solvent interactions in the electrospinning of polyacrylonitrile/iron acetylacetonate. *Journal of Applied Polymer Science*, 109(5), 2935–2941. <https://doi.org/10.1002/APP.28396>
- Duan, M., Sun, J., Huang, Y., Jiang, H., Hu, Y., Pang, J., & Wu, C. (2023). Electrospun gelatin/chitosan nanofibers containing curcumin for multifunctional food packaging. *Food Science and Human Wellness*, 12(2), 614–621. <https://doi.org/10.1016/j.fshw.2022.07.064>
- Duan, M., Yu, S., Sun, J., Jiang, H., Zhao, J., Tong, C., Hu, Y., Pang, J., & Wu, C. (2021). Development and characterization of electrospun nanofibers based on pullulan/chitin nanofibers containing curcumin and anthocyanins for active-intelligent food packaging. *International Journal of Biological Macromolecules*, 187, 332–340. <https://doi.org/10.1016/J.IJBIOMAC.2021.07.140>
- Ehrmann, A. (2021). Non-Toxic Crosslinking of Electrospun Gelatin Nanofibers for Tissue Engineering and Biomedicine—A Review. *Polymers 2021*, Vol. 13, Page 1973, 13(12), 1973. <https://doi.org/10.3390/POLYM13121973>
- El-Sakhawy, M., & Elshakankery, M. (2017). Technology of nano-fibers: Production techniques and properties-Critical review Eco-Friendly Composite Material from Agricultural Waste View project Water and wastewater treatment

View project. In *Article in Journal of the Textile Association*.
<https://www.researchgate.net/publication/322774945>

- Emir, A. A., Yildiz, E., Aydogdu, Y., & Sumnu, G. (2023). Active Films Based on Faba Bean (*Vicia faba* L.) Flour Incorporated with Sumac (*Rhus coriaria*): Assessment of Antioxidant and Antimicrobial Performances of Packaging for Shelf Life of Chicken Breast. *Food and Bioprocess Technology*, *16*(2), 327–341. <https://doi.org/10.1007/s11947-022-02940-y>
- Erdem, R., & Akalin, M. (2015). Characterization and evaluation of antimicrobial properties of electrospun chitosan/polyethylene oxide based nanofibrous scaffolds (with/without nanosilver). *Journal of Industrial Textiles*, *44*(4), 553–571. <https://doi.org/10.1177/1528083713503000>
- Etxabide, A., Coma, V., Guerrero, P., Gardrat, C., & de la Caba, K. (2017). Effect of cross-linking in surface properties and antioxidant capacity of gelatin films incorporated with a curcumin derivative. *Food Hydrocolloids*, *66*, 168–175. <https://doi.org/10.1016/j.foodhyd.2016.11.036>
- Fernandez, A., Torres-Giner, S., & Lagaron, J. M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloids*. <https://doi.org/10.1016/j.foodhyd.2008.10.011>
- Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, *40*(16), 4585–4592. [https://doi.org/10.1016/S0032-3861\(99\)00068-3](https://doi.org/10.1016/S0032-3861(99)00068-3)
- García, M. A., Martino, M. N., & Zaritzky, N. E. (2000). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science*, *65*(6), 941–944. <https://doi.org/10.1111/j.1365-2621.2000.tb09397.x>
- Gómez-Estaca, J., Balaguer, M. P., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2017). Improving antioxidant and antimicrobial properties of curcumin by means of encapsulation in gelatin through electrohydrodynamic

- atomization. *Food Hydrocolloids*, 70, 313–320.
<https://doi.org/10.1016/j.foodhyd.2017.04.019>
- Gómez-Estaca, J., Gavara, R., & Hernández-Muñoz, P. (2015). Encapsulation of curcumin in electrosprayed gelatin microspheres enhances its bioaccessibility and widens its uses in food applications. *Innovative Food Science and Emerging Technologies*, 29, 302–307. <https://doi.org/10.1016/j.ifset.2015.03.004>
- Gómez-Estaca, J., López De Lacey, A., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2010). *Biodegradable gelatinechitosan films incorporated with essential oils as antimicrobial agents for fish preservation*. <https://doi.org/10.1016/j.fm.2010.05.012>
- Haug, I. J., & Draget, K. I. (2009). 6 - Gelatin. *Handbook of Hydrocolloids*, 142–163.
- Hettiarachchi, S. S., Perera, Y., Dunuweera, S. P., Dunuweera, A. N., Rajapakse, S., & Rajapakse, R. M. G. (2022). Comparison of Antibacterial Activity of Nanocurcumin with Bulk Curcumin. *ACS Omega*, 7(50), 46494–46500. <https://doi.org/10.1021/acsomega.2c05293>
- Himesh, S., Sita Sharan, P., Govind, N., Soni, H., & Professor, A. (2011). QUALITATIVE AND QUANTITATIVE PROFILE OF CURCUMIN FROM ETHANOLIC EXTRACT OF CURCUMA LONGA. In *IRJP* (Vol. 2, Issue 4). <http://www.irjponline.com>
- Hsu, C. M., & Shivkumar, S. (2004). Nano-sized beads and porous fiber constructs of Poly(ϵ -caprolactone) produced by electrospinning. *Journal of Materials Science*, 39(9), 3003–3013. <https://doi.org/10.1023/B:JMSC.0000025826.36080.CF/METRICS>
- Huang, X., Jiang, W., Zhou, J., Yu, D. G., & Liu, H. (2022). The Applications of Ferulic-Acid-Loaded Fibrous Films for Fruit Preservation. *Polymers*, 14(22). <https://doi.org/10.3390/polym14224947>

- Hwang, S. W., & Shin, J. S. (2018). Pectin-coated curcumin-chitosan microparticles crosslinked with Mg²⁺ for delayed drug release in the digestive system. *International Journal of Polymer Science*, 2018. <https://doi.org/10.1155/2018/2071071>
- Ibrahim, I. D., Hamam, Y., Sadiku, E. R., Ndambuki, J. M., Kupolati, W. K., Jamiru, T., Eze, A. A., & Snyman, J. (2022). Need for Sustainable Packaging: An Overview. In *Polymers* (Vol. 14, Issue 20). MDPI. <https://doi.org/10.3390/polym14204430>
- Jadhav, N. R., Gaikwad, V. L., Nair, K. J., & Kadam, H. M. (2009). Glass transition temperature: Basics and application in pharmaceutical sector. In *Asian Journal of Pharmaceutics* (Vol. 3, Issue 2, pp. 82–89). <https://doi.org/10.4103/0973-8398.55043>
- Khalid Saeed, M., Ahmad, I., Hina, S., Zahra, N., Kalim, I., Masood, S., & Syed, Q. (n.d.). *Physico-chemical Analysis, Total Polyphenolic Content and Antioxidant Capacity of Yellow Dye Extracted from Curcuma longa*.
- Ki, C. S., Baek, D. H., Gang, K. D., Lee, K. H., Um, I. C., & Park, Y. H. (2005). Characterization of gelatin nanofiber prepared from gelatin-formic acid solution. *Polymer*, 46(14), 5094–5102. <https://doi.org/10.1016/j.polymer.2005.04.040>
- Kidoaki, S., Kwon, I. K., & Matsuda, T. (2006). Structural features and mechanical properties of in situ-bonded meshes of segmented polyurethane electrospun from mixed solvents. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials*, 76(1), 219–229. <https://doi.org/10.1002/JBM.B.30336>
- Kilic, B., Dogan, V., Kilic, V., & Kahyaoglu, L. N. (2022). Colorimetric food spoilage monitoring with carbon dot and UV light reinforced fish gelatin films using a smartphone application. *International Journal of Biological Macromolecules*, 209, 1562–1572. <https://doi.org/10.1016/J.IJBIOMAC.2022.04.119>

- Kotatha, D., Hirata, M., Ogino, M., Uchida, S., Ishikawa, M., Furuike, T., & Tamura, H. (2019). Preparation and Characterization of Electrospun Gelatin Nanofibers for Use as Nonaqueous Electrolyte in Electric Double-Layer Capacitor. *Journal of Nanotechnology*, 2019. <https://doi.org/10.1155/2019/2501039>
- Kumar, R., Ghoshal, • G, & Goyal, • M. (2019). Synthesis and functional properties of gelatin/CA-starch composite film: excellent food packaging material. *J Food Sci Technol.*, 56(4), 1954–1965. <https://doi.org/10.1007/s13197-019-03662-4>
- Lawhavinit, O.-A., Kongkathip, N., & Kongkathip, B. (2010). Antimicrobial Activity of Curcuminoids from *Curcuma longa* L. on Pathogenic Bacteria of Shrimp and Chicken. In *Nat. Sci.* (Vol. 44).
- Le Salem, M., Mauguen, Y., & Prangé, T. (2010). Structural Biology and Crystallization Communications Revisiting glutaraldehyde cross-linking: the case of the Arg-Lys intermolecular doublet. *Structural Communications Acta Cryst*, 66, 225–228. <https://doi.org/10.1107/S1744309109054037>
- Liu, S., Luo, S., Li, Y., Zhang, H., Yuan, Z., Shang, L., & Deng, L. (2023). Influence of the Maillard Reaction on Properties of Air-Assisted Electrospun Gelatin/Zein/Glucose Nanofibers. *Foods*, 12(3). <https://doi.org/10.3390/foods12030451>
- Loganathan, S., Valapa, R. B., Mishra, R. K., Pugazhenti, G., & Thomas, S. (2017). Thermogravimetric Analysis for Characterization of Nanomaterials. In *Thermal and Rheological Measurement Techniques for Nanomaterials Characterization* (Vol. 3, pp. 67–108). Elsevier. <https://doi.org/10.1016/B978-0-323-46139-9.00004-9>
- López-De-Dicastillo, C., Pezo, D., Nerín, C., López-Carballo, G., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2012). Reducing Oxidation of Foods Through Antioxidant Active Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids. *Packaging Technology and Science*, 25(8), 457–466. <https://doi.org/10.1002/PTS.992>

- Luca, A., Cilek, B., Hasirci, V., Sahin, S., & Sumnu, G. (2013). Effect of Degritting of Phenolic Extract from Sour Cherry Pomace on Encapsulation Efficiency-Production of Nano-suspension. *Food and Bioprocess Technology*, 6(9), 2494–2502. <https://doi.org/10.1007/s11947-012-0880-z>
- MALI K. K., DHAWALE S. C., DIAS R. J., DHANE N. S., & GHORPADE V. S. (2018). *Citric Acid Crosslinked Carboxymethyl Cellulose-based Composite Hydrogel Films for Drug Delivery*. www.ijpsonline.com
- Marcos, B., Sárraga, C., Castellari, M., Kappen, F., Schennink, G., & Arnau, J. (2014). Development of biodegradable films with antioxidant properties based on polyesters containing α -tocopherol and olive leaf extract for food packaging applications. *Food Packaging and Shelf Life*, 1(2), 140–150. <https://doi.org/10.1016/J.FPSL.2014.04.002>
- Martins, N., Roriz, C. L., Morales, P., Barros, L., & Ferreira, I. C. F. R. (2016). Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. In *Trends in Food Science and Technology* (Vol. 52, pp. 1–15). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2016.03.009>
- Miletić, A., Pavlić, B., Ristić, I., Zeković, Z., & Pilić, B. (2019). Encapsulation of Fatty Oils into Electrospun Nanofibers for Cosmetic Products with Antioxidant capacity. *Applied Sciences* 2019, Vol. 9, Page 2955, 9(15), 2955. <https://doi.org/10.3390/APP9152955>
- Mohammad, S., Dadfar, M., Kavooosi, G., & Dadfar, A. (2013). Investigation of Mechanical Properties, Antibacterial Features, and Water Vapor Permeability of Polyvinyl Alcohol Thin Films Reinforced by Glutaraldehyde and Multiwalled Carbon Nanotube. *Polymer Composites*. <https://doi.org/10.1002/pc.22827>
- Morota, K., Matsumoto, H., Mizukoshi, T., Konosu, Y., Minagawa, M., Tanioka, A., Yamagata, Y., & Inoue, K. (2004). Poly(ethylene oxide) thin films produced by

- electrospray deposition: Morphology control and additive effects of alcohols on nanostructure. *Journal of Colloid and Interface Science*, 279(2), 484–492. <https://doi.org/10.1016/j.jcis.2004.06.075>
- Musso, Y. S., Salgado, P. R., & Mauri, A. N. (2017). Smart edible films based on gelatin and curcumin. *Food Hydrocolloids*, 66, 8–15. <https://doi.org/10.1016/j.foodhyd.2016.11.007>
- Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). *Food Chemistry*, 86(3), 325–332. <https://doi.org/10.1016/j.foodchem.2003.09.038>
- Niamsa, N., & Sittiwet, C. (2004). *Antimicrobial Activity of Curcuma longa Aqueous Extract*.
- Ninan, G., Joseph, J., & Aliyamveetil, Z. A. (2014). A comparative study on the physical, chemical and functional properties of carp skin and mammalian gelatins. *Journal of Food Science and Technology*, 51(9), 2085–2091. <https://doi.org/10.1007/s13197-012-0681-4>
- Olde Damink, L. H. H., Dijkstra, P. J., Van Luyn, M. J. A., Van Wachem, P. B., Nieuwenhuis, P., & Feijen, J. (1995). Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. *Journal of Materials Science: Materials in Medicine*, 6(8), 460–472. <https://doi.org/10.1007/BF00123371/METRICS>
- Oliveira Filho, J. G. de, Bertolo, M. R. V., Rodrigues, M. Á. V., Marangon, C. A., Silva, G. da C., Odoni, F. C. A., & Egea, M. B. (2021). Curcumin: A multifunctional molecule for the development of smart and active biodegradable polymer-based films. In *Trends in Food Science and Technology* (Vol. 118, pp. 840–849). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2021.11.005>
- PAKOLPAKÇIL, A. (2022). Effect of Glutaraldehyde Crosslinking Parameters on Mechanical and Wetting Properties of PVA/NaAlg Electrospun Mat. *Sakarya*

University Journal of Science, 26(5), 990–999.
<https://doi.org/10.16984/laufenbilder.1089304>

- Park, H. Y., Kim, S. J., Kim, K. M., You, Y. S., Kim, S. Y., & Han, J. (2012). Development of Antioxidant Packaging Material by Applying Corn-Zein to LLDPE Film in Combination with Phenolic Compounds. *Journal of Food Science*, 77(10), E273–E279. <https://doi.org/10.1111/J.1750-3841.2012.02906.X>
- Pavoni, J. M. F., dos Santos, N. Z., May, I. C., Pollo, L. D., & Tessaro, I. C. (2021). Impact of acid type and glutaraldehyde crosslinking in the physicochemical and mechanical properties and biodegradability of chitosan films. *Polymer Bulletin*, 78(2), 981–1000. <https://doi.org/10.1007/s00289-020-03140-4>
- Priyadarshi, R., Sauraj, Kumar, B., & Negi, Y. S. (2018). Chitosan film incorporated with citric acid and glycerol as an active packaging material for extension of green chilli shelf life. *Carbohydrate Polymers*, 195, 329–338. <https://doi.org/10.1016/j.carbpol.2018.04.089>
- Priyadarsini, K. I., Maity, D. K., Naik, G. H., Kumar, M. S., Unnikrishnan, M. K., Satav, J. G., & Mohan, H. (2003). Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant capacity of curcumin. *Free Radical Biology and Medicine*, 35(5), 475–484. [https://doi.org/10.1016/S0891-5849\(03\)00325-3](https://doi.org/10.1016/S0891-5849(03)00325-3)
- Prystupa, D. A., & Donald, A. M. (1996). Infrared study of gelatin conformations in the gel and sol states. In *Polymer Gels and Networks* (Vol. 4).
- Qiao, C., Ma, X., Wang, X., & Liu, L. (2021). Structure and properties of chitosan films: Effect of the type of solvent acid. *LWT*, 135. <https://doi.org/10.1016/j.lwt.2020.109984>
- Ramakrishna, S., Fujihara, K., Teo, W. E., Yong, T., Ma, Z., & Ramaseshan, R. (2006). Electrospun nanofibers: solving global issues. *Materials Today*, 9(3), 40–50.

- Ramos, M., Valdés, A., Beltrán, A., & Garrigós, M. (2016). Gelatin-Based Films and Coatings for Food Packaging Applications. *Coatings*, 6(4), 41. <https://doi.org/10.3390/coatings6040041>
- Rawdkuen, S., Sai-Ut, S., & Benjakul, S. (2010). Properties of gelatin films from giant catfish skin and bovine bone: A comparative study. *European Food Research and Technology*, 231(6), 907–916. <https://doi.org/10.1007/s00217-010-1340-5>
- Robb, B., & Lennox, B. (2011). The electrospinning process, conditions and control. *Electrospinning for Tissue Regeneration*, 51–66. <https://doi.org/10.1533/9780857092915.1.51>
- Roy, S., & Rhim, J. W. (2020). Preparation of carbohydrate-based functional composite films incorporated with curcumin. *Food Hydrocolloids*, 98. <https://doi.org/10.1016/j.foodhyd.2019.105302>
- Russell, D. A. M. (2014). Sustainable (food) packaging - an overview. *Food Additives and Contaminants - Part A*, 31(3), 396–401. <https://doi.org/10.1080/19440049.2013.856521>
- Said, N. S., & Sarbon, N. M. (2022). Physical and Mechanical Characteristics of Gelatin-Based Films as a Potential Food Packaging Material: A Review. In *Membranes* (Vol. 12, Issue 5). MDPI. <https://doi.org/10.3390/membranes12050442>
- Salgado, P. R., López-Caballero, M. E., Gómez-Guillén, M. C., Mauri, A. N., & Montero, M. P. (2012). Exploration of the antioxidant and antimicrobial capacity of two sunflower protein concentrate films with naturally present phenolic compounds. *Food Hydrocolloids*, 29(2), 374–381. <https://doi.org/10.1016/j.foodhyd.2012.03.006>
- Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of*

Agricultural and Food Chemistry, 53(20), 7749–7759.
<https://doi.org/10.1021/jf051513y>

Sharma, L., Sharma, H. K., & Saini, C. S. (2018). Edible films developed from carboxylic acid cross-linked sesame protein isolate: barrier, mechanical, thermal, crystalline and morphological properties. *Journal of Food Science and Technology*, 55(2), 532–539. <https://doi.org/10.1007/s13197-017-2962-4>

Silva De Campos, S., De Oliveira, A., Fernandes, T., Moreira, M., Vieira Da Silva, T. B., Vieira Da Silva, M., Pinto, J. A., Bilck, A. P., Hess Gonçalves, O., Fernandes, I. P., Barreiro, M.-F., Yamashita, F., Valderrama, P., Shirai, M. A., & Leimann, F. V. (2019). *TPCS/PBAT blown extruded films added with curcumin as a technological approach for active packaging materials*. <https://doi.org/10.1016/j.fpsl.2019.100424>

Siracusa, V., & Rosa, M. D. (2018). 8 SUSTAINABLE PACKAGING. *Sustainable Food Systems from Agriculture to Industry*. <https://doi.org/10.1016/B978-0-12-811935-8.00008-1>

Solghi, S., Emam-Djomeh, Z., Fathi, M., & Farahani, F. (2020). The encapsulation of curcumin by whey protein: Assessment of the stability and bioactivity. *Journal of Food Process Engineering*, 43(6). <https://doi.org/10.1111/jfpe.13403>

Soradech, S., Nunthanid, J., Limmatvapirat, S., & Luangtana-Anan, M. (2012). An approach for the enhancement of the mechanical properties and film coating efficiency of shellac by the formation of composite films based on shellac and gelatin. *Journal of Food Engineering*, 108(1), 94–102. <https://doi.org/10.1016/J.JFOODENG.2011.07.019>

Suderman, N., Isa, M. I. N., & Sarbon, N. M. (2018). Characterization on the mechanical and physical properties of chicken skin gelatin films in comparison to mammalian gelatin films. *IOP Conference Series: Materials Science and Engineering*, 440(1). <https://doi.org/10.1088/1757-899X/440/1/012033>

- Tam, N., Oguz, S., Aydogdu, A., Sumnu, G., & Sahin, S. (2017). Influence of solution properties and pH on the fabrication of electrospun lentil flour/HPMC blend nanofibers. *Food Research International*, *102*, 616–624. <https://doi.org/10.1016/j.foodres.2017.09.049>
- Tangkanakul, P., Auttaviboonkul, P., Niyomwit, B., Lowvitoon, N. , Charoenthamawat, P., & Trakoontivakorn, G. (2009). Antioxidant capacity, total phenolic content and nutritional composition. *International Food Research Journal*, *16*, 571–580.
- Tyagi, P., Singh, M., Kumari, H., Kumari, A., & Mukhopadhyay, K. (2015). Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. *PLoS ONE*, *10*(3). <https://doi.org/10.1371/journal.pone.0121313>
- Uranga, J., Nguyen, B. T., Si, T. T., Guerrero, P., & De la Caba, K. (2020). The effect of cross-linking with citric acid on the properties of agar/fish gelatin films. *Polymers*, *12*(2). <https://doi.org/10.3390/polym12020291>
- Valizadeh, S., Naseri, M., Babaei, S., Hosseini, S. M. H., & Imani, A. (2019). Development of bioactive composite films from chitosan and carboxymethyl cellulose using glutaraldehyde, cinnamon essential oil and oleic acid. *International Journal of Biological Macromolecules*, *134*, 604–612. <https://doi.org/10.1016/j.ijbiomac.2019.05.071>
- Vass, P., Szabó, E., Domokos, A., Hirsch, E., Galata, D., Farkas, B., Démuth, B., Andersen, S. K., Vigh, T., Verreck, G., Marosi, G., & Nagy, Z. K. (2020). Scale-up of electrospinning technology: Applications in the pharmaceutical industry. In *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* (Vol. 12, Issue 4). Wiley-Blackwell. <https://doi.org/10.1002/wnan.1611>
- Walsh, H., & Kerry, J. P. (2012). Packaging of ready-to-serve and retail-ready meat, poultry and seafood products. *Advances in Meat, Poultry and Seafood Packaging*, 406–436. <https://doi.org/10.1533/9780857095718.3.406>

- Williams, G. R., Raimi-Abraham, B. T., & Luo, C. J. (2021). *Chapter Title: Electrospinning fundamentals Book Title: Nanofibres in Drug Delivery*. <https://about.jstor.org/terms>
- Williamson, G. (2017). The role of polyphenols in modern nutrition. In *Nutrition Bulletin* (Vol. 42, Issue 3, pp. 226–235). Blackwell Publishing Ltd. <https://doi.org/10.1111/nbu.12278>
- Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant capacity and phenolic compounds in 32 selected herbs. *Food Chemistry*, *105*(3), 940–949. <https://doi.org/10.1016/j.foodchem.2007.04.038>
- Wong, C. C., Li, H. Bin, Cheng, K. W., & Chen, F. (2006). A systematic survey of antioxidant capacity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry*, *97*(4), 705–711. <https://doi.org/10.1016/J.FOODCHEM.2005.05.049>
- Wu, H., Lei, Y., Lu, J., Zhu, R., Xiao, D., Jiao, C., Xia, R., Zhang, Z., Shen, G., Liu, Y., Li, S., & Li, M. (2019). Effect of citric acid induced crosslinking on the structure and properties of potato starch/chitosan composite films. *Food Hydrocolloids*, *97*. <https://doi.org/10.1016/j.foodhyd.2019.105208>
- Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. In *Chemical Reviews* (Vol. 119, Issue 8, pp. 5298–5415). American Chemical Society. <https://doi.org/10.1021/acs.chemrev.8b00593>
- Yang, Z., Peng, H., Wang, W., & Liu, T. (2010). Crystallization behavior of poly(ϵ -caprolactone)/layered double hydroxide nanocomposites. *Journal of Applied Polymer Science*, NA-NA. <https://doi.org/10.1002/app.31787>
- Yıkar, e., demir, d., & bölgen, n. (2021). Electrospinning of gelatin nanofibers: effect of gelatin concentration on chemical, morphological and degradation characteristics. *Turkish journal of engineering*. <https://doi.org/10.31127/tuje.704573>

- Yildirim, S., Röcker, B., Pettersen, K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., & Coma, V. (2017). Active Packaging Applications for Food. *Comprehensive Reviews in Food Science and Food Safety*, *17*, 165–199. <https://doi.org/10.1111/1541-4337.12322>
- Yildiz, E., Aydogdu Emir, A., Sumnu, G., & Kahyaoglu, L. N. (2022). Citric acid cross-linked curcumin/chitosan/chickpea flour film: An active packaging for chicken breast storage. *Food Bioscience*, *50*, 2212–4292. <https://doi.org/10.1016/j.fbio.2022.102121>
- Yildiz, E., Ilhan, E., Kahyaoglu, L. N., Sumnu, G., & Oztop, M. H. (2022). The effects of crosslinking agents on faba bean flour–chitosan–curcumin films and their characterization. *Legume Science*, *4*(1). <https://doi.org/10.1002/leg3.121>
- Yildiz, E., Sumnu, G., & Kahyaoglu, L. N. (2021). Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. *International Journal of Biological Macromolecules*, *170*, 437–446. <https://doi.org/10.1016/j.ijbiomac.2020.12.160>
- Yildiz, E., Sumnu, G., & Kahyaoglu, L. N. (2022). Assessment of curcumin incorporated chickpea flour/PEO (polyethylene oxide) based electrospun nanofiber as an antioxidant and antimicrobial food package. *Food and Bioproducts Processing*, *135*, 205–216. <https://doi.org/10.1016/j.fbp.2022.08.002>
- Zeren, S., Sahin, S., & Sumnu, G. (2022). Encapsulation of Caffeic Acid in Carob Bean Flour and Whey Protein-Based Nanofibers via Electrospinning. *Foods*, *11*(13). <https://doi.org/10.3390/foods11131860>
- Zhang, A., Han, Y., & Zhou, Z. (2023). Characterization of citric acid crosslinked chitosan/gelatin composite film with enterocin CHQS and red cabbage pigment. *Food Hydrocolloids*, *135*, 108144. <https://doi.org/10.1016/J.FOODHYD.2022.108144>

Zhao, L., Duan, G., Zhang, G., Yang, H., Jiang, S., & He, S. (2020). Electrospun functional materials toward food packaging applications: A review. In *Nanomaterials* (Vol. 10, Issue 1). MDPI AG. <https://doi.org/10.3390/nano10010150>

APPENDICES

A. Statistical Analysis

Table A. 1 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for power law index (n) values of the film forming solutions

■ RHEOLOGY N K

One-way ANOVA: n versus Solution

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$
Rows unused	4

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	0.001215	0.000405	1.72	0.300
Error	4	0.000942	0.000235		
Total	7	0.002157			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0153425	56.34%	23.60%	0.00%

Means

Solution	N	Mean	StDev	95% CI
GL	2	0.98795	0.00600	(0.95783, 1.01808)
GLC	2	0.9532	0.0243	(0.9231, 0.9833)
GLCCA0.5	2	0.96876	0.00310	(0.93863, 0.99888)
GLCCA1	2	0.9684	0.0175	(0.9383, 0.9986)

Pooled StDev = 0.0153425

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GL	2	0.98795	A
GLCCA0.5	2	0.96876	A
GLCCA1	2	0.9684	A
GLC	2	0.9532	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 consistency coefficient (k) values of the film forming solutions

RHEOLOGY N K

One-way ANOVA: k versus Solution

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Rows unused 4

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	0.097543	0.032514	74.09	0.001
Error	4	0.001755	0.000439		
Total	7	0.099298			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0209484	98.23%	96.91%	92.93%

Means

Solution	N	Mean	StDev	95% CI
GL	2	0.4401	0.0220	(0.3989, 0.4812)
GLC	2	0.24579	0.00596	(0.20467, 0.28692)
GLCCA0.5	2	0.17486	0.00873	(0.13373, 0.21599)
GLCCA1	2	0.1654	0.0341	(0.1243, 0.2065)

Pooled StDev = 0.0209484

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GL	2	0.4401	A
GLC	2	0.24579	B
GLCCA0.5	2	0.17486	B
GLCCA1	2	0.1654	B

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 average viscosity values of the film forming solutions

One-way ANOVA: viscosity versus Solution

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$
Rows unused	4

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	0.103606	0.034535	237.92	0.000
Error	4	0.000581	0.000145		
Total	7	0.104187			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0120480	99.44%	99.02%	97.77%

Means

Solution	N	Mean	StDev	95% CI
GL	2	0.41433	0.00827	(0.39068, 0.43798)
GLC	2	0.16786	0.01172	(0.14420, 0.19151)
GLCCA0.5	2	0.14931	0.01019	(0.12565, 0.17296)
GLCCA1	2	0.1404	0.0165	(0.1167, 0.1640)

Pooled StDev = 0.0120480

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GL	2	0.41433	A
GLC	2	0.16786	B
GLCCA0.5	2	0.14931	B
GLCCA1	2	0.1404	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 electrical conductivity values of the film forming solutions

One-way ANOVA: Electrical Conductivity versus Solution

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
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	3.33989	1.11330	35979.22	0.000
Error	4	0.00012	0.00003		
Total	7	3.34001			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0055626	100.00%	99.99%	99.99%

Means

Solution	N	Mean	StDev	95% CI
GL	2	2.74533	0.00189	(2.73441, 2.75625)
GLC	2	1.30757	0.00316	(1.29665, 1.31849)
GLCCA0.5	2	1.29360	0.00481	(1.28268, 1.30452)
GLCCA1	2	1.17300	0.00933	(1.16208, 1.18392)

Pooled StDev = 0.00556262

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GL	2	2.74533	A
GLC	2	1.30757	B
GLCCA0.5	2	1.29360	B
GLCCA1	2	1.17300	C

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 Fiber Size Diameter values of the films

One-way ANOVA: FSD versus Films

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$
Rows unused	4

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Films	4	FFS0, FFS1, FFS2, GL

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Films	3	98418.0	32806.0	40387.24	0.000
Error	4	3.2	0.8		
Total	7	98421.3			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.901269	100.00%	99.99%	99.99%

Means

Films	N	Mean	StDev	95% CI
FFS0	2	182.246	0.619	(180.477, 184.016)
FFS1	2	344.033	0.304	(342.264, 345.803)
FFS2	2	427.417	1.390	(425.647, 429.186)
GL	2	162.420	0.916	(160.651, 164.190)

Pooled StDev = 0.901269

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Films	N	Mean	Grouping
FFS2	2	427.417	A
FFS1	2	344.033	B
FFS0	2	182.246	C
GL	2	162.420	D

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 L values of the film forming solutions

One-way ANOVA: L versus Solution

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
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	92.5120	30.8373	2005.68	0.000
Error	4	0.0615	0.0154		
Total	7	92.5735			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.123996	99.93%	99.88%	99.73%

Means

Solution	N	Mean	StDev	95% CI
GL	2	90.6350	0.0071	(90.3916, 90.8784)
GLC	2	82.0350	0.0778	(81.7916, 82.2784)
GLCCA0.5	2	83.120	0.198	(82.877, 83.363)
GLCCA1	2	83.4800	0.1273	(83.2366, 83.7234)

Pooled StDev = 0.123996

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GL	2	90.6350	A
GLCCA1	2	83.4800	B
GLCCA0.5	2	83.120	B
GLC	2	82.0350	C

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 L values of the films

One-way ANOVA: L versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	67.2501	22.4167	283.08	0.000
Error	4	0.3167	0.0792		
Total	7	67.5669			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.281403	99.53%	99.18%	98.12%

Means

Film	N	Mean	StDev	95% CI
GL	2	19.255	0.290	(18.703, 19.807)
GLC	2	13.915	0.262	(13.363, 14.467)
GLCCA0.5	2	20.605	0.403	(20.053, 21.157)
GLCCA1	2	14.5400	0.0424	(13.9875, 15.0925)

Pooled StDev = 0.281403

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLCCA0.5	2	20.605	A
GL	2	19.255	B
GLCCA1	2	14.5400	C
GLC	2	13.915	C

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 a values of the film forming solutions

One-way ANOVA: a versus Solution

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
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	78.5346	26.1782	1173.25	0.000
Error	4	0.0893	0.0223		
Total	7	78.6239			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.149374	99.89%	99.80%	99.55%

Means

Solution	N	Mean	StDev	95% CI
GL	2	-3.980	0.226	(-4.273, -3.687)
GLC	2	4.3500	0.0283	(4.0567, 4.6433)
GLCCA0.5	2	2.4300	0.0283	(2.1367, 2.7233)
GLCCA1	2	2.205	0.191	(1.912, 2.498)

Pooled StDev = 0.149374

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GLC	2	4.3500	A
GLCCA0.5	2	2.4300	B
GLCCA1	2	2.205	B
GL	2	-3.980	C

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 a values of the films

One-way ANOVA: a versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	47.4219	15.8073	181.67	0.000
Error	4	0.3480	0.0870		
Total	7	47.7700			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.294979	99.27%	98.72%	97.09%

Means

Film	N	Mean	StDev	95% CI
GL	2	-1.190	0.141	(-1.769, -0.611)
GLC	2	5.330	0.382	(4.751, 5.909)
GLCCA0.5	2	3.350	0.382	(2.771, 3.929)
GLCCA1	2	3.845	0.191	(3.266, 4.424)

Pooled StDev = 0.294979

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLC	2	5.330	A
GLCCA1	2	3.845	B
GLCCA0.5	2	3.350	B
GL	2	-1.190	C

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 b values of the film forming solutions

One-way ANOVA: b versus Solution

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
Solution	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solution	3	2427.71	809.237	14119.74	0.000
Error	4	0.23	0.057		
Total	7	2427.94			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.239400	99.99%	99.98%	99.96%

Means

Solution	N	Mean	StDev	95% CI
GL	2	17.330	0.325	(16.860, 17.800)
GLC	2	56.8650	0.0495	(56.3950, 57.3350)
GLCCA0.5	2	57.790	0.156	(57.320, 58.260)
GLCCA1	2	57.990	0.311	(57.520, 58.460)

Pooled StDev = 0.239400

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solution	N	Mean	Grouping
GLCCA1	2	57.990	A
GLCCA0.5	2	57.790	A B
GLC	2	56.8650	B
GL	2	17.330	C

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 b values of the films

One-way ANOVA: b versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	149.686	49.8954	424.55	0.000
Error	4	0.470	0.1175		
Total	7	150.156			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.342819	99.69%	99.45%	98.75%

Means

Film	N	Mean	StDev	95% CI
GL	2	1.200	0.226	(0.527, 1.873)
GLC	2	8.345	0.304	(7.672, 9.018)
GLCCA0.5	2	13.170	0.339	(12.497, 13.843)
GLCCA1	2	9.325	0.460	(8.652, 9.998)

Pooled StDev = 0.342819

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLCCA0.5	2	13.170	A
GLCCA1	2	9.325	B
GLC	2	8.345	B
GL	2	1.200	C

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 Antioxidant capacity values (AA%) of the films

One-way ANOVA: AA% versus Film

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
Film	4	GL, GLC, GLCC0.5, GLCC1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	1256.44	418.814	363.49	0.000
Error	4	4.61	1.152		
Total	7	1261.05			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.07341	99.63%	99.36%	98.54%

Means

Film	N	Mean	StDev	95% CI
GL	2	-0.398	0.282	(-2.505, 1.709)
GLC	2	32.01	1.88	(29.90, 34.11)
GLCC0.5	2	22.466	0.625	(20.359, 24.573)
GLCC1	2	27.934	0.781	(25.826, 30.041)

Pooled StDev = 1.07341

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLC	2	32.01	A
GLCC1	2	27.934	A
GLCC0.5	2	22.466	B
GL	2	-0.398	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 Total Phenolic Content values of the films

One-way ANOVA: TPC versus Film

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
Film	3	GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	2	0.008635	0.004317	19.57	0.019
Error	3	0.000662	0.000221		
Total	5	0.009296			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0148515	92.88%	88.14%	71.53%

Means

Film	N	Mean	StDev	95% CI
GLC	2	0.35495	0.00700	(0.32153, 0.38837)
GLCCA0.5	2	0.3228	0.0175	(0.2894, 0.3562)
GLCCA1	2	0.2634	0.0175	(0.2299, 0.2968)

Pooled StDev = 0.0148515

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLC	2	0.35495	A
GLCCA0.5	2	0.3228	A B
GLCCA1	2	0.2634	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 Encapsulation Efficiency values of the films

One-way ANOVA: EE versus Film

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
Film	3	GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	2	252.49	126.244	19.57	0.019
Error	3	19.35	6.450		
Total	5	271.84			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.53960	92.88%	88.14%	71.53%

Means

Film	N	Mean	StDev	95% CI
GLC	2	60.696	1.197	(54.981, 66.411)
GLCCA0.5	2	55.19	2.99	(49.48, 60.91)
GLCCA1	2	45.04	2.99	(39.32, 50.75)

Pooled StDev = 2.53960

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLC	2	60.696	A
GLCCA0.5	2	55.19	A B
GLCCA1	2	45.04	B

Means that do not share a letter are significantly different.

Table A. 15 One-way Analysis of Variance (ANOVA) and Tukey’s comparison test for Weight Loss 1 (TGA1) values of the films

One-way ANOVA: TGA1 versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	17.200	5.733	5.38	0.025
Error	8	8.526	1.066		
Total	11	25.726			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.03234	66.86%	54.43%	25.43%

Means

Film	N	Mean	StDev	95% CI
GL	3	11.110	0.433	(9.736, 12.484)
GLC	3	10.200	1.714	(8.826, 11.574)
GLCCA0.5	3	8.763	0.912	(7.389, 10.138)
GLCCA1	3	8.047	0.553	(6.672, 9.421)

Pooled StDev = 1.03234

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	3	11.110	A
GLC	3	10.200	A B
GLCCA0.5	3	8.763	A B
GLCCA1	3	8.047	B

Means that do not share a letter are significantly different.

Table A. 16 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Weight Loss 2 (TGA2) values of the films

One-way ANOVA: TGA2 versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	10.242	3.4141	10.72	0.004
Error	8	2.548	0.3185		
Total	11	12.790			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.564343	80.08%	72.61%	55.18%

Means

Film	N	Mean	StDev	95% CI
GL	3	61.430	0.252	(60.679, 62.181)
GLC	3	60.9600	0.1153	(60.2086, 61.7114)
GLCCA0.5	3	60.907	0.555	(60.155, 61.658)
GLCCA1	3	63.180	0.943	(62.429, 63.931)

Pooled StDev = 0.564343

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLCCA1	3	63.180	A
GL	3	61.430	B
GLC	3	60.9600	B
GLCCA0.5	3	60.907	B

Means that do not share a letter are significantly different.

Table A. 17 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Crystallinity % (XRD) values of the films

One-way ANOVA: Crystallinity versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	33.610	11.2033	17.69	0.009
Error	4	2.534	0.6334		
Total	7	36.143			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.795862	92.99%	87.73%	71.96%

Means

Film	N	Mean	StDev	95% CI
GL	2	2.220	0.252	(0.658, 3.782)
GLC	2	1.626	0.315	(0.063, 3.188)
GLCCA0.5	2	6.50	1.54	(4.94, 8.06)
GLCCA1	2	1.5625	0.0898	(0.0000, 3.1250)

Pooled StDev = 0.795862

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLCCA0.5	2	6.50	A
GL	2	2.220	B
GLC	2	1.626	B
GLCCA1	2	1.5625	B

Means that do not share a letter are significantly different.

Table A. 18 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Glass Transition Temperature (Tg) (DSC) values of the films

One-way ANOVA: Tg versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	3150.94	1050.31	514.69	0.000
Error	4	8.16	2.04		
Total	7	3159.10			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.42852	99.74%	99.55%	98.97%

Means

Film	N	Mean	StDev	95% CI
GL	2	69.16	1.46	(66.36, 71.97)
GLC	2	119.730	1.117	(116.925, 122.535)
GLCCA0.5	2	112.73	1.64	(109.93, 115.53)
GLCCA1	2	110.49	1.44	(107.69, 113.29)

Pooled StDev = 1.42852

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLC	2	119.730	A
GLCCA0.5	2	112.73	B
GLCCA1	2	110.49	B
GL	2	69.16	C

Means that do not share a letter are significantly different.

Table A. 19 One-way Analysis of Variance (ANOVA) and Tukey’s comparison test for Melting Temperature Start (Tm Start) (DSC) values of the films

One-way ANOVA: Tm Start versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	556.14	185.381	28.33	0.004
Error	4	26.17	6.543		
Total	7	582.31			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.55788	95.51%	92.13%	82.02%

Means

Film	N	Mean	StDev	95% CI
GL	2	55.10	1.57	(50.08, 60.12)
GLC	2	48.98	2.93	(43.96, 54.00)
GLCCA0.5	2	32.35	2.80	(27.33, 37.37)
GLCCA1	2	46.56	2.70	(41.54, 51.58)

Pooled StDev = 2.55788

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	2	55.10	A
GLC	2	48.98	A
GLCCA1	2	46.56	A
GLCCA0.5	2	32.35	B

Means that do not share a letter are significantly different.

Table A. 20 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Melting Temperature Peak (T_m Peak) (DSC) values of the films

One-way ANOVA: T_m Peak versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	45.786	15.262	14.36	0.013
Error	4	4.251	1.063		
Total	7	50.037			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.03084	91.51%	85.13%	66.02%

Means

Film	N	Mean	StDev	95% CI
GL	2	80.285	0.728	(78.261, 82.309)
GLC	2	73.835	1.308	(71.811, 75.859)
GLCCA0.5	2	78.055	1.407	(76.031, 80.079)
GLCCA1	2	76.010	0.170	(73.986, 78.034)

Pooled StDev = 1.03084

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	2	80.285	A
GLCCA0.5	2	78.055	A B
GLCCA1	2	76.010	B C
GLC	2	73.835	C

Means that do not share a letter are significantly different.

Table A. 21 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Melting Temperature End (Tm End) (DSC) values of the films

One-way ANOVA: Tm End versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	2460.2	820.07	28.12	0.004
Error	4	116.7	29.17		
Total	7	2576.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.40066	95.47%	92.08%	81.89%

Means

Film	N	Mean	StDev	95% CI
GL	2	106.36	4.40	(95.76, 116.96)
GLC	2	98.52	8.79	(87.91, 109.12)
GLCCA0.5	2	142.69	3.20	(132.09, 153.29)
GLCCA1	2	103.25	3.14	(92.65, 113.85)

Pooled StDev = 5.40066

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GLCCA0.5	2	142.69	A
GL	2	106.36	B
GLCCA1	2	103.25	B
GLC	2	98.52	B

Means that do not share a letter are significantly different.

Table A. 22 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Degradation Temperature (Tdeg) (DSC) values of the films

One-way ANOVA: T deg versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	35.65	11.884	4.57	0.088
Error	4	10.41	2.603		
Total	7	46.06			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.61331	77.40%	60.45%	9.60%

Means

Film	N	Mean	StDev	95% CI
GL	2	231.515	1.068	(228.348, 234.682)
GLC	2	227.745	1.280	(224.578, 230.912)
GLCCA0.5	2	230.14	2.57	(226.97, 233.31)
GLCCA1	2	226.050	1.004	(222.883, 229.217)

Pooled StDev = 1.61331

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	2	231.515	A
GLCCA0.5	2	230.14	A
GLC	2	227.745	A
GLCCA1	2	226.050	A

Means that do not share a letter are significantly different.

Table A. 23 One-way Analysis of Variance (ANOVA) and Tukey’s comparison test for Water Vapor Permeability (WVP) values of the films

One-way ANOVA: WVP versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	0.000000	0.000000	24.23	0.005
Error	4	0.000000	0.000000		
Total	7	0.000000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0000000	94.79%	90.87%	79.14%

Means

Film	N	Mean	StDev	95% CI
GL	2	0.000000	0.000000	(0.000000, 0.000000)
GLC	2	0.000000	0.000000	(0.000000, 0.000000)
GLCCA0.5	2	0.000000	0.000000	(0.000000, 0.000000)
GLCCA1	2	0.000000	0.000000	(0.000000, 0.000000)

Pooled StDev = 3.598437E-11

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	2	0.000000	A
GLCCA1	2	0.000000	A B
GLC	2	0.000000	B C
GLCCA0.5	2	0.000000	C

Means that do not share a letter are significantly different.

Table A. 24 One-way Analysis of Variance (ANOVA) and Tukey's comparison test for Thickness values of the films

One-way ANOVA: Thickness (mm) versus Film

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
Film	4	GL, GLC, GLCCA0.5, GLCCA1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Film	3	0.002021	0.000674	27.00	0.004
Error	4	0.000100	0.000025		
Total	7	0.002120			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0049945	95.29%	91.76%	81.18%

Means

Film	N	Mean	StDev	95% CI
GL	2	0.08763	0.00348	(0.07782, 0.09743)
GLC	2	0.05858	0.00813	(0.04878, 0.06839)
GLCCA0.5	2	0.04950	0.00377	(0.03969, 0.05931)
GLCCA1	2	0.08225	0.00271	(0.07244, 0.09206)

Pooled StDev = 0.00499454

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Film	N	Mean	Grouping
GL	2	0.08763	A
GLCCA1	2	0.08225	A
GLC	2	0.05858	B
GLCCA0.5	2	0.04950	B

Means that do not share a letter are significantly different.

B. Calibration Curves

Table B. 1 Calibration curve for Antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

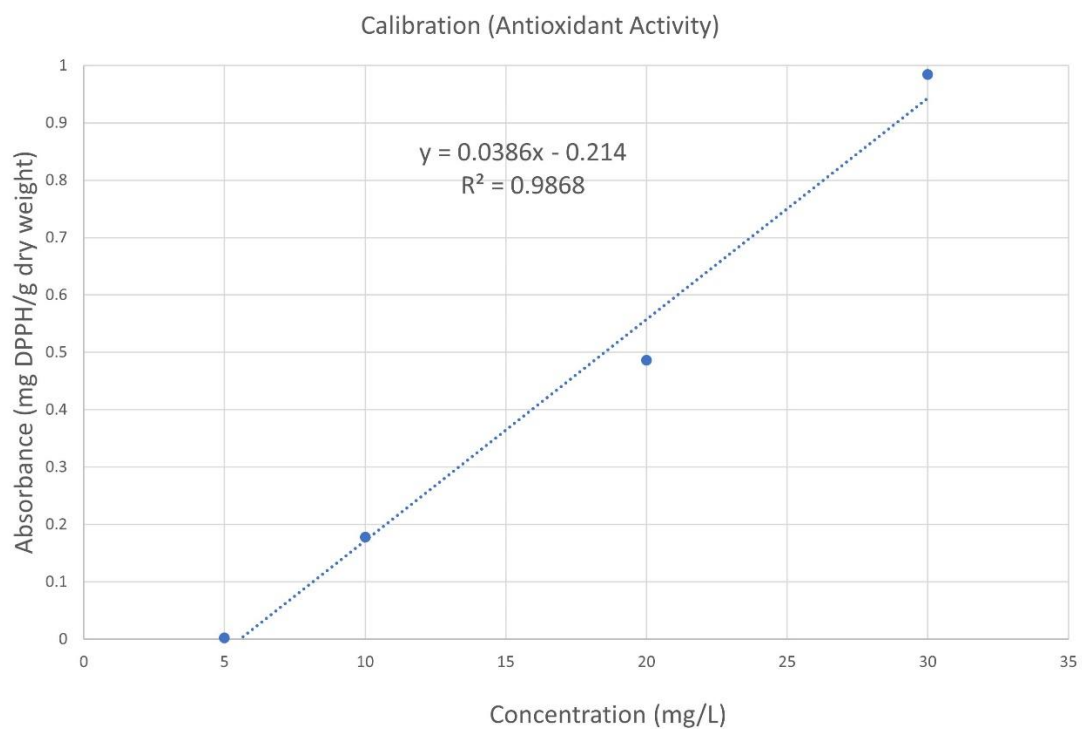


Table B. 2 Calibration curve for Total Phenolic Content using Gallic Acid

