

Biotechnological Production and Characterization of Innovative Antimicrobial and Antioxidant Edible Films Enriched with Safflower (*Carthamus tinctorius* L.) Extract

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Research Article

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Abstract

Background

Sustainable and functional alternatives to petroleum-based plastics are urgently needed in food biotechnology. Biopolymer-based edible films, particularly those enriched with bioactive plant extracts, provide innovative solutions for extending shelf life while reducing environmental impact. Safflower (*Carthamus tinctorius* L.), rich in phenolics and flavonoids, offers strong antimicrobial and antioxidant potential for active packaging development.

Methods

Edible films were prepared using whey protein isolate with varying concentrations of safflower extract. Physicochemical, thermal, and structural properties were examined by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). Antimicrobial activity was tested against *Escherichia coli* and *Candida albicans*. Moisture retention and solubility assays were performed to evaluate stability and biodegradability.

Results

The highest extract concentration (EF4) showed the strongest inhibition against both microorganisms. TGA confirmed biodegradability with ~ 90% total weight loss, while DSC demonstrated distinct thermal transitions indicating stability. FTIR verified successful incorporation of hydroxyl, carbonyl, and glycerol-derived groups. Higher film-forming volumes improved moisture retention, whereas high solubility indicated rapid degradability.

Conclusions

Safflower-enriched films present a biotechnological innovation in sustainable food packaging. The integration of bioactive plant compounds into whey protein-based matrices yields active films combining antimicrobial and antioxidant activity with environmental compatibility. These findings highlight their potential as next-generation materials in food biotechnology, with future studies needed for scale-up and industrial validation.

1. INTRODUCTION

Food biotechnology has increasingly focused on the development of bio-based packaging materials as sustainable alternatives to synthetic plastics. The incorporation of plant-derived bioactive compounds into protein- or polysaccharide-based matrices represents a biotechnological innovation that enables functionalization of edible films with antimicrobial and antioxidant activities (Campos et al., 2024; Zhang

et al., 2023). Unlike conventional plastics, these films can be engineered through bioprocessing approaches that not only provide barrier properties but also contribute to active food protection and environmental sustainability (Mellinas et al., 2016; Versino et al., 2023).

In this context, safflower (*Carthamus tinctorius* L.), a medicinal plant rich in phenolic compounds and flavonoids, is an attractive candidate for functional biotechnological applications. The integration of safflower extract into whey protein isolate (WPI)-based matrices allows the production of edible films with enhanced bioactivity, combining food biotechnology innovation with environmental biotechnology perspectives (Kim et al., 2015; González et al., 2022). Such dual benefits position safflower-enriched edible films as next-generation materials with potential to reduce plastic waste while improving food preservation.

Food packaging is a technology engineered to extend the shelf life of food products, prevent physical and chemical deterioration, and guarantee hygiene and convenience during transportation (Chauhan et al., 2022; Alves et al., 2022). While conventional packaging materials such as plastic, glass, metal, and paper have long dominated the sector, the persistence of plastic waste in the environment has heightened the importance of biodegradable and sustainable alternatives (Sastre et al., 2022; Versino et al., 2023; Foods, 2025).

The advancement of packaging technologies has progressed beyond merely safeguarding food, leading to the emergence of smart and active packaging systems. Active packaging directly interacts with the food product to extend its shelf life and maintain quality by incorporating components such as oxygen scavengers, antimicrobial agents, and antioxidants. These systems are designed to absorb or release substances that control the internal environment of the package, thereby inhibiting spoilage while maintaining the food's sensory and nutritional qualities (Jiang et al., 2023; Vilela et al., 2018). In contrast, smart packaging consists of systems integrated with sensors and indicators that monitor environmental conditions, offering consumers real-time information about the product's quality and safety (Ahmed et al., 2022; Liu et al., 2021). The implementation of these technologies facilitates more effective monitoring of food shelf life and contributes to the reduction of waste.

In recent years, edible films thin biopolymer-based layers have gained prominence within active packaging, as they can be applied directly onto food surfaces or used as standalone packaging materials (Campos et al., 2024; Susmita Devi et al., 2024; Polymer Bulletin, 2025). These films are synthesized from natural polymers, including proteins, polysaccharides, and lipids, thereby offering a sustainable alternative for prolonging food shelf life and minimizing packaging waste (Mellinas et al., 2016).

1.1. Materials utilized in the preparation of edible films

Edible film coatings are categorized into polysaccharides, proteins, lipids, or composites based on their constituent components. Polysaccharide-based films exhibit favorable mechanical resistance and gas

barrier properties; however, their hydrophilic nature makes them susceptible to moisture, potentially compromising their integrity (Hashemi et al., 2023; Zhao et al., 2021). Protein-based films, typically formulated from solutions or dispersions, demonstrate high flexibility but are subject to limitations such as moisture susceptibility and potential allergic reactions (Chen et al., 2019; Mihalca et al., 2021). Lipid-based coatings are primarily employed to establish moisture barriers; yet, they possess certain drawbacks related to their brittle structure and waxy taste (Devi et al., 2024; Usman et al., 2025). Composite films are produced by blending different polymers with the aim of enhancing mechanical strength and barrier properties for improved food packaging performance (Liu et al., 2023; Zhang and Kumar, 2024).

1.2. Application methods of edible films

Edible films are applied to the surface of food products utilizing various techniques to establish a protective barrier. These methods encompass dipping, spraying, pouring, extrusion, and brushing (Krochta and De Mulder-Johnston, 1997). Dipping consists of immersing the food product into an edible film or coating solution, allowing for uniform coverage, which is especially beneficial for products with irregular or complex surfaces (Souza et al., 2022; Ahmed et al., 2021). The spraying technique entails the application of the film solution onto the surface in a thin layer and is predominantly favored for fruits, vegetables, and meat products (Cerqueira et al., 2017). The pouring method involves spreading the edible film solution over a surface followed by drying, rendering it suitable for the production of independent films intended as packaging materials (Kester and Fennema, 1986). The extrusion method is executed by processing film-forming materials under conditions of heat and pressure, facilitating large-scale production within the food industry (Mellinas et al., 2016). The brushing technique, commonly employed in small-scale applications, involves the manual application of edible films or coatings onto food surfaces using a brush. This method allows for precise control over the application, making it suitable for laboratory-scale experiments and small production batches. Factors such as brush type, bristle material, and application pressure can influence the uniformity and effectiveness of the coating (Wang, 2023; Krishnan et al., 2025). The selection of the appropriate method is contingent upon various factors, including film composition, the specific food product targeted, and the desired barrier properties (Zhao, 2011).

1.3. Safflower and general properties

The safflower plant (*Carthamus tinctorius* L.) is an important crop cultivated for multiple industrial applications, including edible oil production, animal feed, textile dyes, and pharmaceutical products (Abbas et al., 2021; Li et al., 2023). The seeds of safflower (*Carthamus tinctorius*) contain 35–50% oil, of which approximately 90% consists of unsaturated fatty acids (Tahmasebpour et al., 2011; Kiproviski et al., 2021). The fatty acid profile is typically composed of 71–75% linoleic acid (C18:2), 16–20% oleic acid (C18:1), 6–8% palmitic acid (C16:0), and 2–3% stearic acid (C18:0) (Kurt et al., 2025). Linoleic acid is particularly important due to its potential role in reducing cardiovascular disease risk and improving cholesterol levels (Verywell Health, 2024).

The flowers of safflower contain phenolic compounds and flavonoids, which give the plant strong antioxidant properties. Compounds such as gallic acid, chlorogenic acid, quercetin-3-galactoside, and epicatechin are present in safflower flowers, and these compounds may contribute to the prevention of cardiovascular diseases and cancer due to their antioxidant effects (Erbaş et al., 2023; Ren, 2025).

Moreover, the oleic acid-rich variant of safflower oil demonstrates quality comparable to olive oil and shares similar sensory attributes, including aroma, flavor, and appearance, with sunflower oil (González et al., 2022; Patel and Singh, 2023).

2. MATERIAL AND METHOD

2.1. Biotechnological production of edible films

Edible films were produced using a biopolymer-based matrix composed of whey protein isolate (WPI), glycerol as a plasticizer, and safflower (*Carthamus tinctorius L.*) flower extract as a source of bioactive compounds. Film-forming solutions were prepared by dispersing WPI in distilled water under continuous stirring and heating to ensure complete solubilization and protein denaturation. Glycerol was incorporated as a plasticizer to enhance flexibility and reduce brittleness. The bioactive extract was added at different concentrations to achieve films with antimicrobial and antioxidant functionality. The final dispersions were cast onto sterile Petri dishes and dried under controlled temperature and humidity conditions to allow solvent evaporation and film formation. This production process represents a simple, scalable, and environmentally friendly biotechnological approach for developing active edible packaging materials.

Table 1
Composition of Edible Films (EF: Edible Film)

Film composition	Whey isolate (% w/w)	Glycerol (% w/w)	Guargum (% w/w)	Safflower extract (% v/v)
Control	10	5	0.7	-
Ef1	10	5	0.7	0.5
Ef2	10	5	0.7	1
Ef3	10	5	0.7	1.5
Ef4	10	5	0.7	2

2.2. Extraction of safflower bioactive compounds

Safflower petals were dried, ground, and subjected to aqueous-ethanolic extraction. The extract was filtered, concentrated under reduced pressure, and stored at 4°C until use. This extraction step provided phenolic-rich bioactive compounds, which were subsequently integrated into the film matrix to enhance

its biological activity. The incorporation of plant-derived compounds into a protein-based carrier system demonstrates a biotechnological strategy for functional material development.

2.3. Bioactivity assays (antimicrobial and antioxidant evaluation)

The antimicrobial properties of the films were assessed against *Escherichia coli* and *Candida albicans* using the agar diffusion method. Films containing different concentrations of safflower extract were placed on inoculated agar plates, and inhibition zones were measured after incubation. Antioxidant activity was evaluated using the DPPH radical scavenging assay to determine the ability of films to prevent oxidative deterioration. These bioactivity assays allowed for the functional validation of the biotechnological coating system.

2.4. Colony forming unite (cfu)

To assess the microbial inhibition of edible film compositions against *Escherichia coli* and *Candida albicans*, 100 μ L of the stock solution containing approximately 107 CFU was extracted from the cell suspensions and inoculated into conical flasks with a total volume of 100 ml, which contained 20 ml of freshly prepared liquid medium. Following the incubation period, edible film compositions with varying concentrations of Safflower flower extract (EF1, EF2, EF3, and EF4) were introduced into the incubated conical flasks along with 20 μ L of the inoculum. Control group conical flasks were maintained without the addition of Safflower flower extract. After incubating *E. coli* at 37°C and *C. albicans* at 28°C, each sample underwent a serial dilution (10^{-1} to 10^{-4}). For the purpose of quantifying microbial inhibition, 100 μ L from each dilution was transferred to petri dishes containing solid medium to calculate colony forming units (CFU).

2.5. Biotechnological characterization of edible films

The physical and structural properties of the films were analyzed to evaluate their suitability as bio-based packaging materials. Moisture content and solubility tests were performed to determine water interaction properties. Thermal stability was characterized using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), while molecular interactions and structural integrity were investigated through Fourier-transform infrared spectroscopy (FTIR). These advanced characterization techniques provided insights into the stability, hydrogen bonding, and incorporation of bioactive compounds within the protein matrix, highlighting the scientific and technological potential of the developed films.

2.6. Moisture determination

Moisture determination was conducted in accordance with AOAC (1990). Edible film samples, positioned within glass drying containers, were subjected to a drying process for 2 hours at 105 ± 2 °C until a constant weight was achieved. The samples were cut to specified dimensions, weighed using a precision scale, and arranged within the glass drying containers to ensure even distribution. Subsequently, the drying process was prolonged for 24 hours at 105 ± 2 °C. Following this duration, the

quantity of moisture lost by the sample was quantified, and the moisture content was calculated based on the initial weight.

2.7. Solubility in water

The solubility (S) of edible film samples in water is determined by the ratio of the initial dry weight of the samples (A_i) to the final dry weight of the insoluble portion (A_f). Initially, the samples were cut into squares measuring 2×2 cm and subjected to drying at 105°C for 24 hours. Subsequently, the dry weight of each sample was recorded (A_i). These samples were then placed in beakers containing specified volumes of pure water (20 ml and 50 ml), covered, and agitated continuously at 25°C for 24 hours. Following this agitation period, the insoluble film residue remaining in the beaker was dried again at 105°C for an additional 24 hours, and the final dry weight (A_f) was determined. This procedure was conducted in triplicate, and the results were averaged.

The solubility percentage was calculated using the provided formula.

$$\% S = \frac{A_i - A_f}{A_i} \times 100$$

1

2.8. Instrumental analyses

2.7.1. Thermogravimetric analysis (Tga)

Thermogravimetric analysis (TGA) serves as a technique to ascertain the mass loss or gain of materials corresponding to variations in temperature or time. This analytical method quantitatively assesses the changes in the mass of a sample as it undergoes temperature elevation. TGA analyses conducted to evaluate the thermal stability of edible film samples were executed under an air atmosphere. These analyses utilized a Shimadzu TGA-50 analyzer, employing samples of 10 mg for each test. The heating rate was established at 10°C/ min, and the scanning range spanned from 30°C to 700°C. This analytical approach yields insights into the thermal resistance and stability of the films, enabling an understanding of their stability particularly under elevated temperature conditions.

2.7.2. Differential scanning calorimetry (Dsc)

In this investigation, differential scanning calorimetry (DSC) tests were conducted employing a Shimadzu DSC-60 device. The tests were administered under an air atmosphere, with a scanning speed set at 10°C /min. The temperature range was specified from 30°C to 500°C, utilizing a sample weight of 5 mg. This analytical technique effectively assesses the thermal transitions and thermal stability of materials.

2.7.3. Fourier transform infrared spectroscopy (Ftir)

Fourier Transform Infrared Spectroscopy (FTIR) spectra of edible film dispersions were recorded at room temperature. These measurements were performed utilizing a detector within the range of 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} . The ATI UNICAM Systems 2000 Fourier Transform Spectrometer was employed to acquire the spectra. This analysis aimed to investigate the chemical structure and functional groups present in the samples. FTIR spectra represent a widely employed technique for elucidating molecular bonds and structures, thereby providing critical insights to enhance the understanding of the properties of edible film materials.

3. RESULTS AND DISCUSSION

3.1. Film formation

Edible films were successfully formed from whey protein isolate (WPI) enriched with different concentrations of safflower extract. Films obtained from the EF4 formulation (15 ml casting volume, 2% extract) exhibited the most favorable characteristics, including ~ 1 mm thickness and a uniform dark-yellow appearance (Fig. 1). This indicates that higher extract concentrations improve film-forming ability and visual uniformity.

3.2. Antimicrobial activity (cfu) of edible films

The antimicrobial properties of the films against *E. coli* and *C. albicans* are summarized in Table 2. A clear concentration-dependent inhibition trend was observed, with EF4 showing the highest microbial reduction. The incorporation of safflower extract into WPI-based films markedly enhanced their antimicrobial efficacy, most likely due to the phenolic and flavonoid constituents of the extract. Similar antimicrobial improvements have been reported in edible films enriched with plant-derived compounds, highlighting the role of bioactive phytochemicals in suppressing microbial growth (Singh et al., 2022; Zajac et al., 2023).

Table 2
Quantification of microbial inhibition by edible films using the CFU method.

Microorganisms	Edible Film Samples	Serial Dilution			
		10^{-1}	10^{-2}	10^{-3}	10^{-4}
<i>E. Coli</i>	Control	∞	∞	∞	265
	EF1	∞	235	114	76
	EF2	∞	196	101	42
	EF3	∞	∞	168	55
	EF4	144	102	76	23
<i>C. Albicans</i>	Control	∞	∞	202	118
	EF1	∞	182	84	55
	EF2	∞	155	72	44
	EF3	∞	196	115	72
	EF4	205	127	95	56

3.3. Characterization analyses

3.3.1. Moisture retention capacity

Moisture retention capacity increased with higher film-forming volumes (8–15 ml), reaching a maximum of 97.1% in EF4 (Table 3). This is consistent with the hydrophilic nature of protein-based matrices, which exhibit high water-binding capacity (Liu et al., 2023). Enhanced moisture retention is desirable for improving the mechanical stability of edible films during storage and handling.

Table 3
Moisture retention percentage of EC4 film at various concentrations.

Film composition amount (ml)	Sample	Moisture rate (%)
8	1	94.56
	2	95.2
	3	92.4
12	1	95.5
	2	94.6
	3	96.2
15	1	95.8
	2	96.4
	3	97.1

3.3.2. Solubility of edible films in water

All films displayed high solubility in water, with values ranging between 92.5% and 96.1% (Table 4). Film 1 showed nearly complete solubility, while EF4 maintained high but slightly lower solubility. These findings indicate that safflower-enriched WPI films are rapidly degradable in aqueous environments, supporting their potential as environmentally sustainable packaging materials. The results are in agreement with recent reports highlighting the biodegradability of protein- and polysaccharide-based films (Versino et al., 2023).

Table 4
Water Solubility of Film Samples

Sample	Film weight		
	Initial weight: ai (g)	Final weight: af (g)	Solubility in water (%)
1	0.130	0.005	96.1
2	0.127	0.007	94.4
3	0.135	0.010	92.5

3.4. Instrumental analyses

3.4.1. Tga results

Thermogravimetric analysis (TGA) was employed to investigate the thermal behavior of the material. The weight loss curve presented in Fig. 2 illustrates the physical or chemical changes occurring at various temperature ranges. Figure 2 provides a detailed account of these weight losses, including percentage values and corresponding temperature ranges. This analysis offers comprehensive insights into the decomposition mechanisms and thermal stability of the material.

The TGA analysis elucidated the decomposition mechanisms of the material across different temperature ranges. The initial weight loss of % 9.985 was ascribed to the presence of moisture or volatile components, whereas the subsequent losses were linked to the degradation of the main structure, as well as organic and inorganic components. The total weight loss, approximately %90, suggests that the material is biodegradable and demonstrates typical thermal behavior.

3.4.2. Differential scanning calorimetry (DSC)

DSC analysis identified three major thermal transitions (Fig. 3). The first minor transition (22.53 J/g) occurred at 60–105°C and was attributed to glass transition phenomena. A stronger exothermic event (105.90 J/g) was detected between 105–196°C, corresponding to partial crystallization or protein denaturation. A major thermal event (672.94 J/g) occurred between 196–375°C, associated with structural decomposition. The multi-stage transitions demonstrate that the films possess adequate thermal stability for practical food packaging applications, in agreement with previous findings for protein-based matrices (Zhang and Kumar, 2024).

3.4.3. Structural analysis (FTIR)

FTIR spectra (Fig. 4) confirmed the incorporation of safflower extract into the WPI film matrix. The broad absorption band at 3283 cm⁻¹ indicated hydroxyl (-OH) groups, associated with phenolic compounds and hydrophilic moieties. The peak at 1638 cm⁻¹ corresponded to carbonyl (C = O) groups from proteins, esters, or aromatic compounds, while bands near 1013 and 950 cm⁻¹ suggested the presence of C–O and C–OH groups, linked to glycerol and carbohydrate structures. These findings confirm that safflower bioactives and glycerol acted synergistically to improve both bioactivity and flexibility of the films (González et al., 2022; Patel and Singh, 2023).

4. DISCUSSION

In this study, biodegradable coating materials were developed by incorporating safflower (*Carthamus tinctorius* L.) flower extract into whey protein isolate (WPI)-based edible films, and their antimicrobial, physicochemical, and structural properties were evaluated. The results indicated that the EF4 formulation, containing the highest extract concentration, exhibited the strongest inhibitory effect against both *Escherichia coli* and *Candida albicans*. The presence of plant-derived phenolic compounds and flavonoids within the film matrix likely played a significant role in suppressing microbial growth. Recent studies have similarly reported that edible films enriched with plant extracts or essential oils can effectively reduce microbial load (Campos et al., 2024; Zhang et al., 2023).

Physicochemical analysis revealed that an increase in the film-forming solution volume significantly enhanced moisture retention capacity, consistent with the hydrophilic nature of protein-based films (Liu et al., 2023). High solubility in water suggests rapid degradability and a strong biodegradability potential from an environmental perspective (Versino et al., 2023). Thermogravimetric analysis (TGA) results indicated approximately 90% weight loss, characteristic of biodegradable materials, while DSC analysis confirmed thermal stability across a wide temperature range with multiple phase transitions (Zhang and Kumar, 2024).

FTIR spectra confirmed the presence of phenolic hydroxyl, carbonyl, and glycerol-derived functional groups in the film matrix, indicating successful incorporation of bioactive and plasticizing components that enhance both biological activity and mechanical flexibility. Recent literature emphasizes that the high phenolic and flavonoid content of safflower extract is a key contributor to its antioxidant and antimicrobial properties (González et al., 2022; Patel and Singh, 2023).

Overall, WPI-based edible films enriched with safflower extract represent a promising, eco-friendly, and functional alternative for active food packaging applications. However, before large-scale industrial application, further research is recommended to include sensory testing in various food systems, long-term storage assessments, and production scale-up studies.

5. CONCLUSION

This study successfully developed whey protein isolate (WPI)-based edible films incorporated with safflower (*Carthamus tinctorius* L.) extract and evaluated their physicochemical, antimicrobial, and structural properties. The highest concentration (EF4) demonstrated the strongest inhibitory effect against *Escherichia coli* and *Candida albicans*, confirming the antimicrobial potential of safflower-derived phenolics and flavonoids. Moisture retention capacity increased with higher film-forming volumes, while high solubility in water confirmed rapid biodegradability, supporting environmental sustainability. Thermal analyses (TGA and DSC) demonstrated multi-stage degradation and high stability across a wide temperature range. FTIR spectra verified the successful incorporation of bioactive and plasticizing components, enhancing both functional and mechanical properties.

Overall, safflower-enriched WPI edible films represent a promising biotechnological alternative to petroleum-based plastics, combining antimicrobial activity with eco-friendly degradation. Future work should focus on sensory evaluation, long-term storage stability, and industrial-scale production to ensure practical applicability in the food industry.

Declarations

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest for this study.

FUNDING DECLARATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

Idea/hypothesis, research, consultant EO; data processing, data analysis, visualisation, writing-review-editing EAG. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Data Availability

The authors confirm that the data supporting the findings of this study are available in the article and supplementary materials. Additional data supporting the findings of this study can be obtained from the corresponding author (E.A.G.) upon reasonable request

Research Involving Plants

All experimental research on plants complied with international guidelines. The safflower (*Carthamus tinctorius* L.) flowers used in this study belong to the Dinçer variety, obtained from the Geçit Kuşağı Agricultural Research Institute (Turkey) and cultivated under controlled greenhouse conditions at Malatya Turgut Özal University. The study did not involve endangered or protected species; therefore, it was not subject to restrictions under the IUCN Policy Statement on Research Involving Species at Risk of Extinction or the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). No special permissions or licenses were required for this research.

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Figures

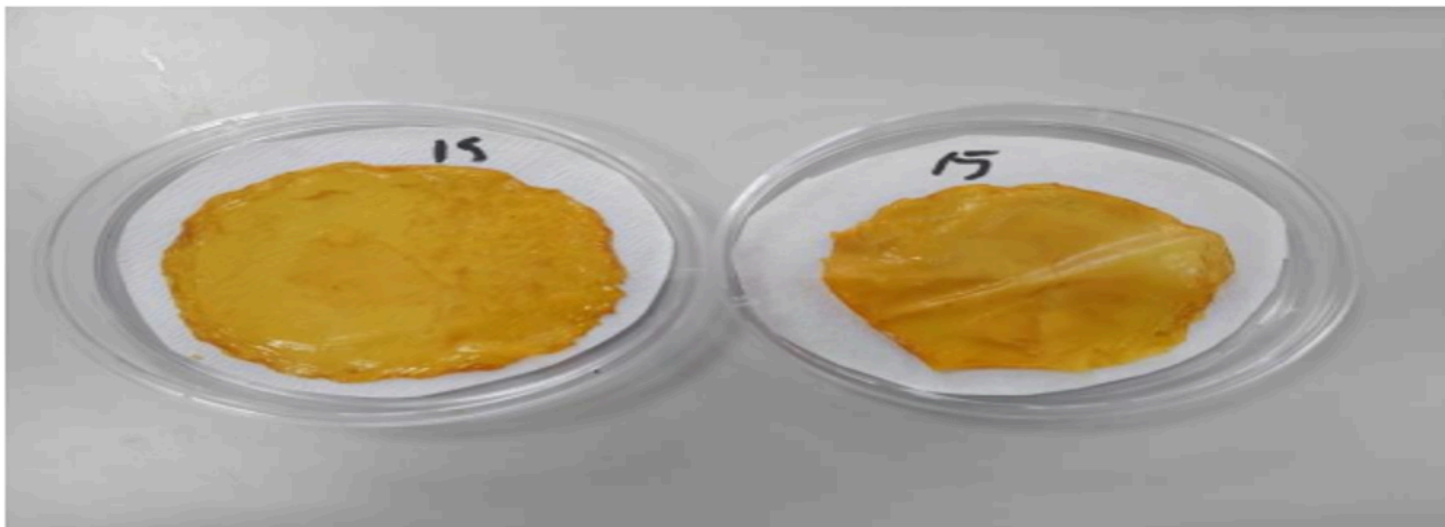


Figure 1

Film samples obtained from EF4 film dispersion

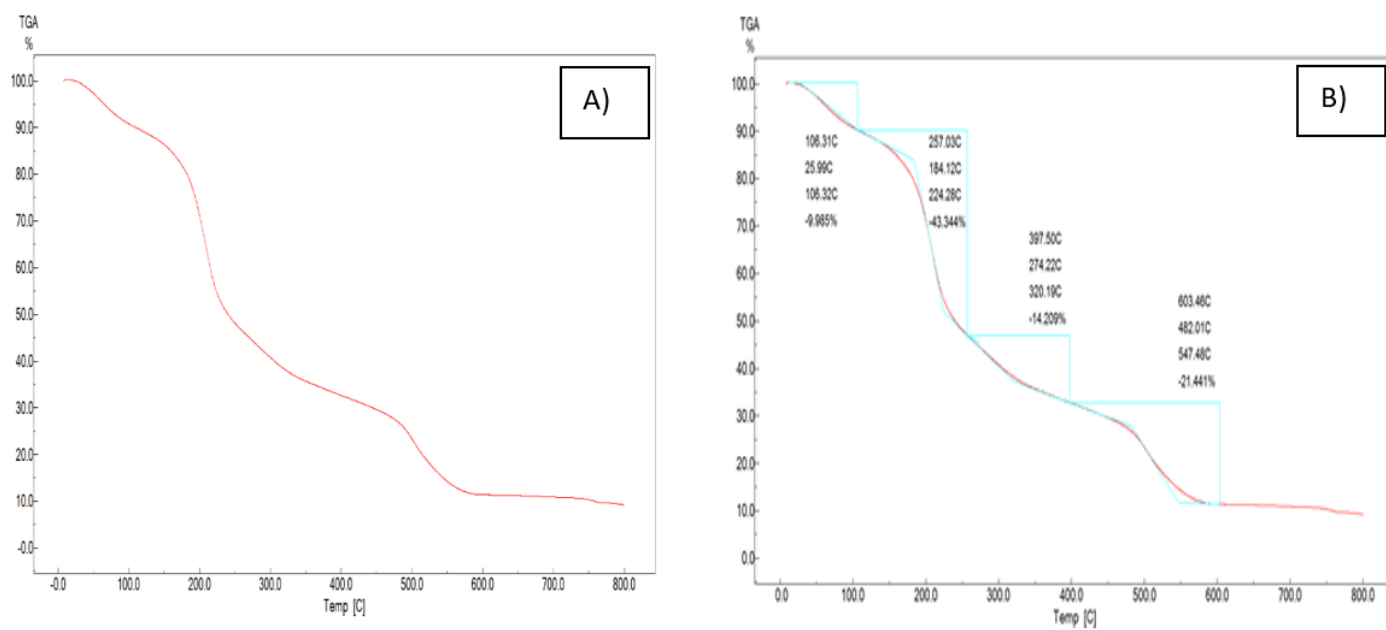


Figure 2

A) and B) Thermogravimetric analysis TGA) graphs of the edible film

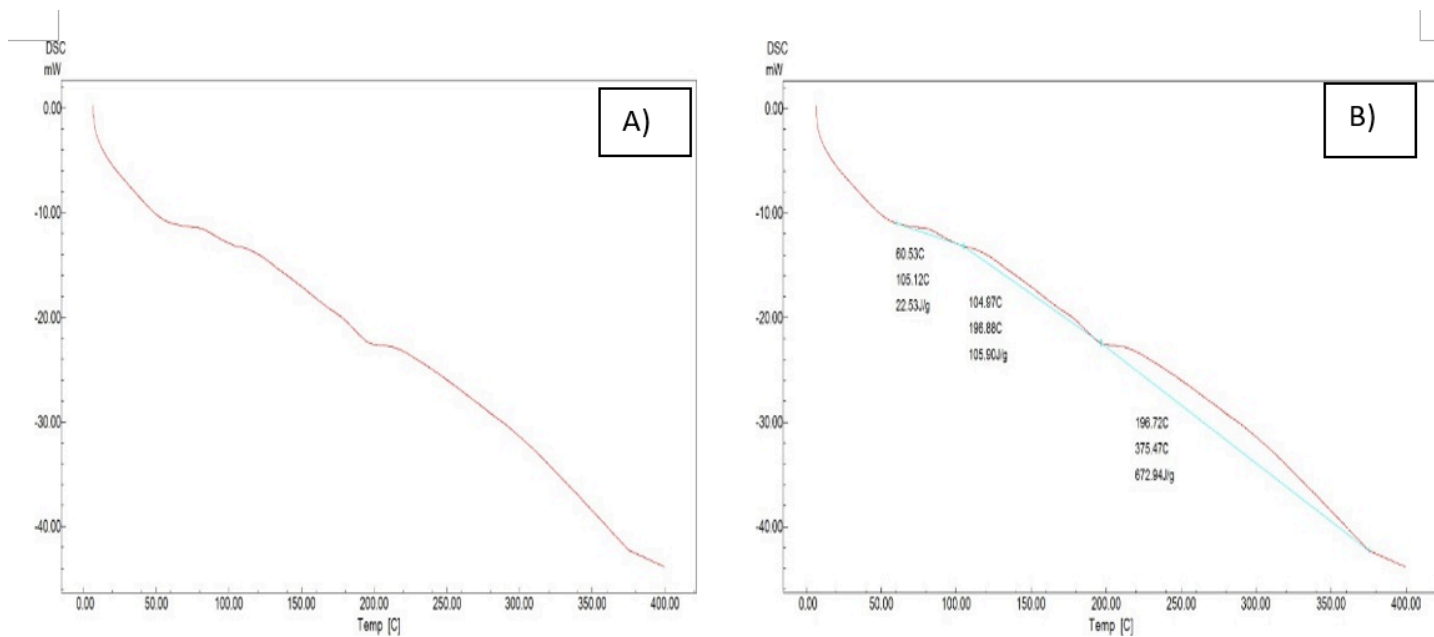


Figure 3

A) and B) Differential Scanning Calorimetry (DSC) analysis graphs of the edible film

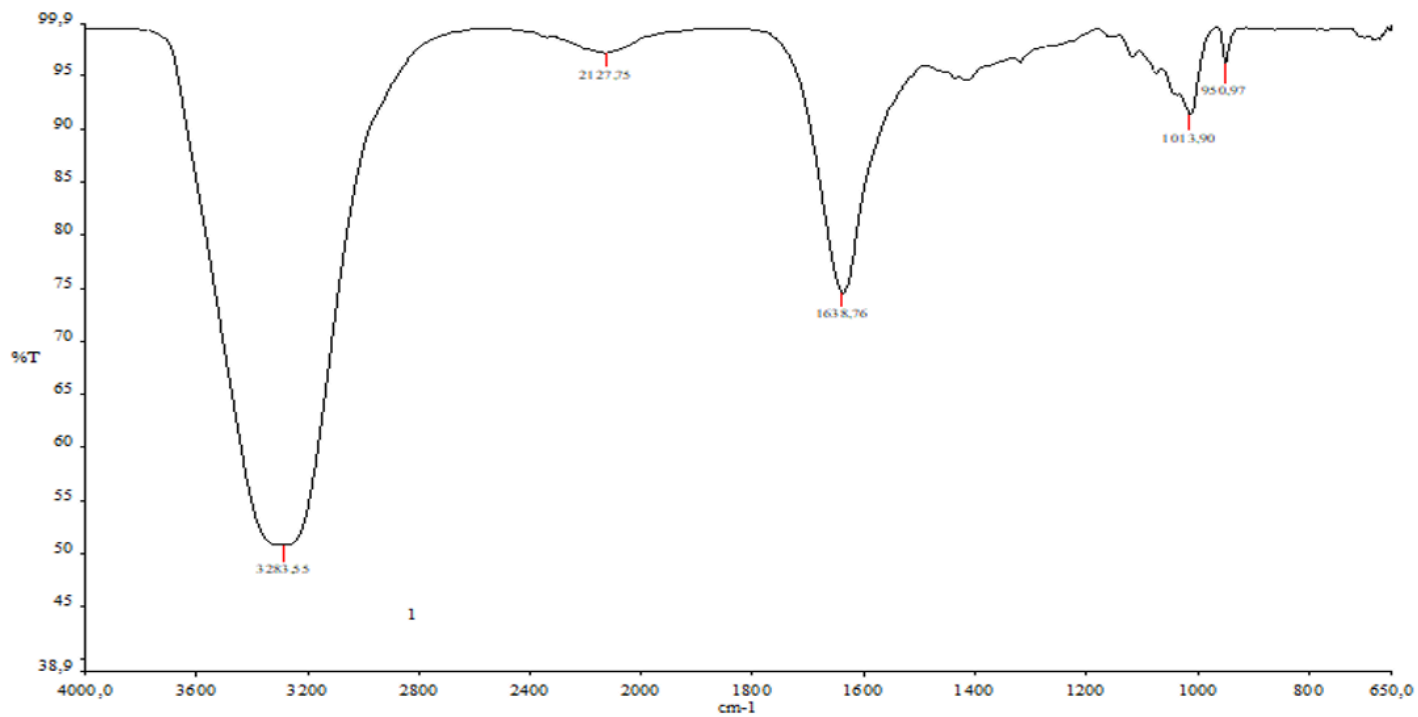


Figure 4

FTIR analysis graph of edible film