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Full Length Research Article

Development of the Lemon Seed Incorporated Edible Film and its Characteristic Assessment

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Abstract

Edible films are type of food packaging created from edible components such as carbohydrates, proteins, lipids, and polysaccharides. They are non-toxic, biodegradable, and compostable. The study's aim is to develop a lemon seed extract incorporated chitosan edible film. It was developed by dissolving deacetylated chitosan flakes and ethanoic lemon seed extract with the aid of glycerol as plasticizer by casting method. The objective is to assess the physical and mechanical properties, such as thickness, tensile strength, elongation at break, wettability, and swelling index of the film. The results revealed that the inclusion of lemon seed extract improved the film thickness, lowered tensile strength while increasing elongation at a break value. The hydrophobicity and swelling index were also reduced. The antioxidant activity was tested using the DPPH, reducing power, and FRAP tests, and lemon seed extract significantly increased the antioxidant activity of the films. Also, as compared to the control, bread packed with the developed film inhibited the proliferation of fungal growth. Our study concluded that the edible films created by using lemon seed extract might be a potential alternative to synthetic materials and as a transport for functional chemicals, adding to biological applications such as food shelf-life extension.

***Key words*:** Edible film, Chitosan, Lemon seed, Food packaging, Antioxidant

Food packaging plays a crucial role in protecting the food chain from chemical, physical, and biological hazards. To protect food from external factors including smells, shocks, dust, temperature, light, and humidity, the packaging is crucial. Food packaging research has gained traction in recent years, owing mostly to customer expectations for food quality and food safety (Kalpana*et al.*, 2019).Food preservation has progressed beyond mere preservation; recent procedures are geared towards achieving two additional goals: the suitability of the applied technologies and the development of ecologically friendly products with no adverse health effects. Additionally, they are seeking extra nutritional qualities. One of these protocols addresses the usage of edible films and coatings. (Díaz-Montes and Castro-Muñoz, 2021)

The development of edible films and coatings has experienced rapid progress in recent decades and is predicted to have a significant influence on food product quality in the next years. (Galus*et al.*, 2020)Edible films and coatings are thin layers that have been used for millennia to preserve food items and keep their components from deteriorating. An edible coating is made directly on the food surface by spraying, dipping, or spreading processes, whereas an edible film is produced first through solvent casting, compression molding, or extrusion techniques and then integrated into food items by being deposited on or between food components. (Ribeiro *et al.*, 2021). Chitosan is the second most prevalent biopolymer on the planet after cellulose. Chitosan is derived from shrimp and other crustaceans' shells. Several extraction procedures have been reported, however, the most commercially viable is chitin deacetylation. (Negm*et al.*, 2020) These polymers can also be extracted using microorganisms in a biological extraction process. The chemical extraction technique for extracting chitin includes the phases of deproteinization, demineralization, and discoloration. (Santos *et al.*, 2020)

Chitosan is a naturally occurring substance that has been used in a variety of applications due to its biocompatibility, biodegradability, and nontoxicity. Chitosan's broad-spectrum antibacterial action lends perfectly to commercialization. (Ke*et al.*, 2021)Chitosan is offered commercially as flakes or powder. It can be used directly or processed into a variety of application modes, including membranes, scaffolds, nanofibers, and micro- and nanospheres(Wang and Zhuang, 2022).

Bhowmik*et al.*, 2022reported that the preservation of perishable foods is greatly aided by novel features such as antibacterial and antioxidant activities as well as the environmentally benign nature of chitosan and its based films. Chitosan film's physical and mechanical qualities are enhanced by the incorporation of essential oils, which also slows the formation of hazardous lipid oxidation products and foodborne bacteria. Plant extracts, particularly essential oils, have lately emerged as viable alternatives to manufactured medications due to their antibacterial, antifungal, anti-inflammatory, antioxidant, and insecticidal activities. (Mele, 2020)Essential oils have demonstrated antiviral activity against a variety of pathogenic viruses. (da Silva *et al.,* 2020) Natural additives, such as essential oils, can be employed in active packaging in the form of films and coatings. (Sharma *et al.,* 2021)

Citrus limonoids (CLs) are a form of highly oxygenated terpenoid secondary metabolite found primarily in the seeds, fruits, and peel tissues of citrus fruits like lemons, limes, oranges, pumellos, grapefruits, bergamots, and mandarins. CL aglycones and glycosides, represented by limonin, have been shown to have a variety of pharmacological activities, including anticancer, antimicrobial, antioxidant, antidiabetic, and insecticidal properties, among others. (Gualdani*et al.,* 2016). Citrus limon L (lemon) is an excellent preventative medicine and in the native medication chest it has a wide range of uses. It can assist in weight loss and reduce the risk of heart disease, anemia, kidney stones, digestive issues, and cancer. (Sm and Rahman, 2019)

The direct use of citrus extracts may cause sensorial problems in the product. Thus, it was intended to develop a new film formulation with better properties containing lemon seed extract that would exhibit reasonable sensory characteristic with extended shelf life.Hence, in this study, lemon seed extract incorporated chitosan-based edible film was formulated and its physical, and antioxidant activity and shelf-life analysis on the quality of bread has been investigated.

MATERIALS AND METHODS

Procurement of raw materials

Chitosan and the chemicalswere procured from Simbioen laboratory in Vandalur, Chennaiand lemon seeds were procured from the local supermarket in Guindy, Chennai, Tamil Nadu, India.

Fig 1 Lemon seed extract incorporated chitosan based edible film

Preparation of lemon seed extract

According to Ucak*et al.,* 2021, the seeds collected from lemon fruits were separated after being pressed. The seeds were then cleaned and dried for two days at 45°C. Later, the lemon seeds had previously been processed into a powder using a blender. 10 g of the powdered lemon seeds was dissolved in 100 ml of 70% ethanol. After 30 minutes of ultrasonic water bath stirring, the liquid was filtered via filter paper. A rotating evaporator was used to evaporate ethanol at 50°C in a vacuum.

Development of lemon seed extract incorporated chitosan film

The chitosan film is developed with the following steps as per Moradi*et al.,* 2012. Chitosan flakes were dissolved in glacial acetic acid. The solution was created with constant stirring overnight at room temperature. It was then filtered through filter paper. Glycerol was then added to the chitosan solution and stirring was continued at room temperature for 30 min. The lemon seed extract was added to the solution. The solutions were placed onto a casting plate. The films were dried for 48 hours at a relative humidity in an incubator room.

Physical properties

Thickness

Film thickness was measured using a digital micro-meter at five different locations of the film and the mean thickness was used to calculate the film properties. (Moradi *et al.,* 2012).

Mechanical properties

Mechanical characteristics [tensile strength (TS) and percentage of elongation at break (%E)] of films were measured according to ASTM D882 (ASTM, 2001) method by using a Universal Testing Instrument Model AI-5000 (Gotech, Taiwan) fitted with a 50 N static load cell.

Wettability of films

This property of films was measured by the sessile drop method using a video-based contact angle meter as mentioned in Moradi *et al.,* 2012. A droplet of distilled water (5 ml) was carefully deposited by a micro-syringe on top of the outer surface of the film and the angle (q) between the film surface and the tangent line at the point of contact of the water droplet with the surface was recorded right after the water drop was deposited. For each film formulation, at least 5 measurements were performed and the average value was obtained.

Swelling index

The water solubility of the chitosan edible film was determined according to the method Emam-Djomeh *et al.,* 2015 with minor modifications. The obtained edible films (25 mm × 20 mm) were immersed in 30 mL of distilled water and continuously stirred for 24 h at room temperature (23 ± 2 ◦C). After this process, the non-solubilized film was taken out, and the solubility of edible films was calculated. The solubility of the edible films was reported as the difference between the initial and final weight. The results were expressed as a percentage and calculated according to the following equation:

$$\text{Water Solubility (\%)} = (Wi - Wf)/Wi \times 100$$

where Wi is the initial weight of the dried film (g), and Wf is the final weight of the dried (immersed) film (g).

Antioxidant activity

DPPH assay

The antioxidant activity of prepared edible films was performed using DPPH (2,2- diphenyl-1-picrylhydrazyl) (G. H. Qiao*et al.,* 2020). Every sample (25 mg) of each film was dissolved in 3 mL of distilled water, and then a 2.8 mL of film extract solution was mixed with 0.2 mL of 1 mM methanolic solutions of DPPH. The absorbance at 517 nm was measured. The percentage of DPPH radical-scavenging activity was calculated according to (Aminzare *et al.,* 2017)using the following equation:

DPPH scavenging effect (%) = $Abs_{DPPH} - Abs_{film\ extract} / Abs_{DPPH} \times 100$

Reducing power assay

Reducing power of chitosan films was carried out according to (Yen & Chen, 1995). Every sample (25 mg) of each film was dissolved in distilled water, and then film extract was mixed with phosphate buffer and potassium ferricyanide. The mixture was incubated at 50°C for 10 min. Trichloroacetic acid was added to the mixture, which was then centrifuged. The upper layer of the solution was mixed with distilled water and FeCl3 and the absorbance was measured at 700 nm. (Moradi*et al.*, 2012).

FRAP assay

A FRAP unit was defined, arbitrarily, as a reduction of 1 mol of Fe (III)to Fe (II). A ferric salt was used as an oxidant. To prepare the FRAP reagent, 2.5 mL of TPTZ was mixed with $FeCl_3$, acetate buffer, and distilled water. A film was placed in 3 mL of FRAP solution and the absorbance at 595 nm was measured 24 h later. The absorbance of the FRAP solution without film was also measured (blank). The antioxidant activity was determined by subtracting the blank from the film absorbance. (Ferreira *et al.*, 2014).

Antifungal activity evaluation

The antifungal activity of the lemon seed extract incorporated chitosan edible films was evaluated by direct contact with bread samples. Bread slices were purchased from a local confectionery store. Uniform bread sampleswere packed and sealed in direct contact with the developed edible film. Samples packed in low-density polyethylene (LDPE) served as the control. All the samples were stored at 24 °C for 10 days. (Tan *et al.*, 2015).

RESULTS AND DISCUSSION

Film thickness

Thickness is an important variable affecting the characteristics of the films, such as tensile strength, elongation, and water vapor permeability. (Sami and Khojah, 2019) Thickness directly affects the appearance of the products, along with their barrier properties against water and gas transmission. G. Qiao *et al.*, 2019 reported that an increasing rate of edible film thickness helps in reducing the diffusion rate.

Thickness has an immediate impact on the appearance of the products, as well as their barrier capabilities against water and gas transfer. The diffusion rate is reduced by increasing the rate of edible film thickness (G. Qiao *et al.*, 2019). The thickness of the film material is directly proportional to the techniques used to prepare it, such as drying, solvent evaporation time, relative humidity, and dish surface (Siracusa *et al.*, 2018).

In this study, the film thickness increased ranging from 0.9 ± 0.1 to 1.2 ± 0.1 mm with an average of 1.06 ± 0.01 as reported in table I due to the addition of glycerol and lemon seed extract.In table 1 a), it is observed that control has 0.07±0.01mm and the sample 1.06±0.01mm with the significant difference ($p<0.05$).

Table 1 Thickness measurement of lemon seed extract incorporated chitosan film

S. No	Thickness 1 (mm)	Thickness 2 (mm)	Thickness 3 (mm)	Thickness 4 (mm)	Thickness 5 (mm)	Average (mm)
1	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.06 ± 0.01

Values represent mean ± standard deviation

Table 1 (a) Thickness measurement – control vs sample

Film formulation	Thickness (mm)
Control – pure chitosan film	0.07±0.01
Sample	1.06 ± 0.01

Values represent mean ± standard deviation

It was observed that films' thickness enhanced with their chitosan content and that it also increased in tandem with their glycerol concentration. Glycerol also contributes to dry matter. This outcome is in line with the results of Nemet*et al.*, 2010. Adding more chitosan to edible film starch-glycerol increased edible film thickness to 0.13 –1.90 mm (Ekariski*et al.*, 2019).

S. Bhuvaneshwari *et al.*, 2011determined that a film formed from 2g chitosan in 2% acetic acid with a thickness of 0.17 mm had a high value when compared to other films. As a result, a 0.17 mm thick chitosan film was used as a test film and reinforced with coconut fiber cellulose to raise the film thickness to 0.32mm.

In Zaman *et al.*, 2018 study, when compared to the film without extract, the film with a greater concentration of Garcinia atroviridis extract created a thicker film ranging from 0.048 to 0.143 mm.

Mechanical properties

Tensile strength is the main indicator of film strength and flexibility. It can be described to the cohesion between the matrices of the film's polymer chains. (Moghadam*et al.*, 2020) Plasticizers in the film formulation have a direct effect on mechanical characteristics because they tend to reduce contacts between neighbouring polymer chains, causing a decrease in tensile strength and an elevation in elongation rate. In other words, plasticizers often aid in increasing the flexibility and elongation of films, but excessive amounts might result in excessive interaction and stiffening of the film. (Matheus*et al.*, 2021).

In this study, the tensile strength of lemon seed extract incorporated chitosan film had decreased with a value of 15.45 MPa ± 1.25 compared to control 16.43Mpa with a significant difference ($p<0.05$) tabulated in Table 2. Pure chitosan film had a tensile strength of 54.9 MPa, while the tensile strength of chitosan-based composite films with 0.5, 1.0 and 1.5% v/v grape seed extract decreased to 20.6, 8.84 and 8.39 MPa, respectively. (Tan *et al.*, 2015).

Similarly, tensile strength of chitosan films with pomegranate peel extract included varied from 32.45 0.98 to 35.23 0.61 MPa. The results reveal that adding pomegranate peel extract has a minimal effect on the films' tensile strength. Several studies have found that incorporating gallic acid to edible films improves their mechanical strength as well as their phenolic and antioxidant activities. (Kumar *et al.*, 2021).

Table 2 Tensile strength and elongation at break of the control (pure chitosan film) and lemon seed extract incorporated chitosan film

Formulation	Tensile strength (Mpa)	Elongation at break (%E)
Control	16.43±0.01	2.143±0.01
Sample	15.45±1.25	7.83±0.76

Values represent mean ± standard deviation

In contrast, the elongation at break values of the developed film increased dramatically when lemon seed extract was added with the value of 7.83±0.76 and the control was 2.143±0.01 with significant difference ($p<0.05$). Elongation at break, also known as fracture strain, is the ratio between the changed length and initial length after the breakage of the test specimen. It expresses the capability of natural plant fiber to resist changes of shape without crack formation. (Djafari Petroudy, 2017). The results of this study were supported by Tan et al., 2015, they mentioned that elongation at break of pure chitosan film was 4.72%, while the elongation at break rose to 56.2, 81.9, and 96.8% for chitosan-based composite films with 0.5, 1.0, and 1.5% v/v grape seed extract, respectively.

In Slimane and Sadok, 2018, Pepsin Solubilized Collagen was combined with chitosan to create a composite film. When compared to chitosan film, this film showed lower tensile strength but increased elongation at break.

Wettability of films

Wettability is an important property of edible films because it allows an understanding of the interfacial film–water interaction and is related to water resistance. (Rotta *et al.*, 2009) One of the simplest ways to describe a material's wettability is to measure the contact angle between water and the film surface. Contact angle values dictate whether a surface is hydrophilic or hydrophobic. Surfaces with values more than 90° are hydrophobic, whereas those with values less than 90° are hydrophilic. (Vilinska, 2009). The mobility of polymeric chains affects physical qualities, which is connected to the nature and concentration of plasticizers, film purity, the hydrophilicity and hydrophobicity ratio of film components, water activities, and film thickness. Water contact angle is commonly used to analyze these qualities. (Matheus *et al.*, 2021)

When distilled water is dropped on the smooth side of the films (the side that supports drying), it does not spread but instead forms an equilibrium spherical cap resting on the film with a contact angle of q < 90, indicating a primarily wetting surface for all chitosan films. Despite its hydrophilic nature, chitosan has water contact angles as high as 53°. (Moradi *et al.*, 2012). The result showed significant difference ($p<0.01$) of the lemon seed extract in the film with a contact angle of 48.11°±1.12 (decrease in hydrophobicity) and the control with 53°±0.1 as shown in (Table 3).

Table 3 Wettability of the control and the developed film

Film formulation	Contact angle
Control – pure chitosan film	53°±0.1
Sample	48.11°±1.12

Values represent mean ± standard deviation

Likewise, Tan *et al.*, 2015 reported that the increased energy of the grape seed extract-incorporated chitosan film flattened the droplet, resulting in a reduced contact angle value (46°).. Pure chitosan film had the highest contact angle and was the most hydrophobic material. The contact angles of composite films decreased significantly as poly-L-lysine concentration increased, with PL-5 film exhibiting the lowest value. This illustrates that blending with poly-L-lysine can increase composite film wettability (Zheng *et al.*, 2009).

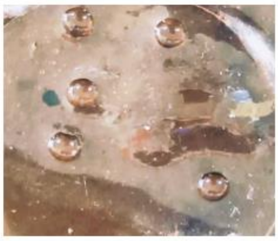

Fig 2 Distilled water Fig 2.1 Contact angle 1

Fig 2.2 Contact angle 2Fig 2.3 Contact angle 3

Swelling index

The swelling capacity of a film indicates its biodegradation and applicability on food products, as well as its water resistance property. (Kumar *et al.*, 2019)According to the findings (Mayachiew and Devahastin, 2010), the rate of swelling of chitosan film is affected by drying temperature as well as the quantity and form of intermolecular chain infarctions.

A greater swelling index may be beneficial in some instances to absorb additional water from the outer surface of moist foods. Moreover, as is well known, the amount and nature of intermolecular chain interactions substantially influence the degree of swelling of a polymeric substance. The film's swelling potential is a critical factor for fresh-cut fruits with high-moisture surfaces. (Kumar *et al.*, 2021)

Srinivasa *et al.*, 2007 mentioned that this property predicts the maintenance of quality during the packaging and storage of food products. The swelling index will decrease and the film thickness will increase with the addition of chitosan and plasticizers. (Nugraheni *et al.*, 2018)

The swelling of pure chitosan film made under ambient conditions was about 173%, due to the hydrophilic property of chitosan which is comparable to the result reported by (de Yao *et al.*, 1996).

In this study, the swelling index of the film is reduced to 26.6 ± 1.05 due to the developed film being incorporated with lemon seed extract as shown in Table 4 and the control with 53°±0.1 with the significant difference ($p<0.05$).

Table 4 Swelling Index of the control and the developed film

Film formulation	Contact angle
Control – pure chitosan film	40%
Sample	26.6%±1.05

Values represent mean ± standard deviation

Fig 3 Before - film in distilled water

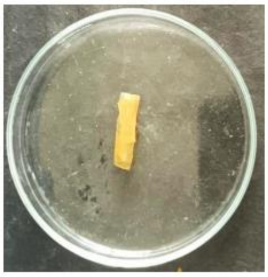

Fig 3.1 After - film in distilled water

When the films were blended with the extract, the swelling of the films was reduced. Although chitosan has hydrophilic groups in its structure, such as carboxylic groups, and these groups could easily interact with water, resulting in film swelling, when enriched with the extract, intermolecular interaction between chitosan and the extract developed, resulting in a decrease in film swelling. Furthermore, the extract's hydrophobic qualities may be responsible for the reduced level of swelling. The extract concentration was shown to have an effect on the swelling ability of antioxidant films. They also reported that due to the hydrophobic nature of the extract and its intermolecular interactions with chitosan, gooseberry extract considerably altered the swelling degree of a chitosan film (Mayachiew and Devahastin, 2010).

Assessment of antioxidant activity of the film

Antioxidant and antimicrobial packaging are two key types of active packaging that show great promise for improving the shelf life of food products. The antioxidant properties of developed chitosan film were examined using free radical-scavenging activity – DPPH assay, reducing power test, and FRAP assays tabulated in Table 5.

Table 5 Antioxidant activities of chitosan film formulated with lemon seed extract

S. No		Day-1	Day-5	Day-10
1	DPPH assay %	63.17 ± 0.01	41.39 ± 0.01	33.31 ± 0.01
2	Reducing power assay %	72.6 ± 0.1	52.01 ± 0.01	43.96 ± 0.01
3	FRAP assay %	69.11 ± 0.01	56.60 ± 0.1	45.98 ± 0.01

Values represent mean ± standard deviation

DPPH assay

Antioxidants are compounds that protect and stabilize the cellular damage brought on by free radicals by giving electrons to the harmed cells. Additionally, antioxidants convert free radicals into waste byproducts that the body excretes. Antioxidant-rich foods are known to reduce the risk of a number of diseases brought on by free radical (Rahman *et al.,* 2015).

A test for measuring antioxidant activity relies on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). It produces a significant absorption maximum at 517 nm due to the existence of an odd electron. The absorption strength decreases as the electron pairs off in the presence of a hydrogen donor, such as an antioxidant that fights free radicals, and the colour change those results is stoichiometric to the quantity of electrons collected (Kommu and Biruduganti, 2019) The efficacy of the films to scavenge DPPH radicals will be determined using the spectrophotometry method on basis of bleaching of the bluish-red or purple colour of DPPH solution as a reagent. (G. H. Qiao*et al.,* 2020)

Results of this study showed that the incorporation of lemon seed extract into chitosan films increased the antioxidant activity of the film up to 63% (on the day of film development) and gradually decreases to 41.39% and 33.31% on the 5th and 10th day respectively as in table 5. Qin *et al.,* 2015showed that chitosan film incorporated with 2 % PRP (pomegranate rind powder) contained the highest amount of total phenolic and was found to be the most active radical scavenger. Moradi *et al.,* 2012 showed chitosan film incorporated with 1% and 2% of Zataria multiflora Boiss essential oils exhibited 33.98% and 37.77% DPPH scavenging activity. Chitosan film incorporated with Eucalyptus globulus essential oil showed that DPPH scavenging activity of 23.03% to 43.62%. (Hafsa*et al.,* 2016).

Reducing power

The principle behind the reducing power assay technique is that compounds with reduction potential combine with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then interacts with ferric chloride to form ferric-ferrous complex with an absorbance maximum at 700 nm. (Bhalodia*et al.,* 2013). This study revealed that the incorporation of lemon seed extract into chitosan films increased the antioxidant activity of the film up to 72.6% (on the day of film development) and gradually decreases to 52.01% and 43.96% on the 5th and 10th day respectively as in table 5. According to Tan *et al.,* 2015 grape seed extract-formulated films have much higher reducing effects than other films.

FRAP Assay

The FRAP assay is a typical electron transfer-based approach for measuring the reduction of ferric ion (Fe3+)-ligand complex to bright blue ferrous (Fe2+). In acidic environments, antioxidants form a complex. Antioxidant activity is measured as an increase in absorbance at 593 nm, and the findings are represented as micromolar Fe2+ equivalents or as a percentage of an antioxidant standard (Antolovich *et al.,* 2002)

The developed film in the present study showed antioxidant activity upto 69.11 (on the day of film development) and reduced to 52.01% and 43.96% on the 5th and 10th day respectively as in (Table 5). The ferric reduction antioxidant activity of chitosan films was modest. The addition of maqui berry extracts to chitosan films improved their antioxidant capabilities relative to the chitosan films, and this improvement was concentration dependent (Genskowsky *et al.,* 2015). According to (Balti *et al.,* 2017) the potential of chitosan film to convert ferric to ferrous iron was extremely limited. The antioxidant capabilities of chitosan films were, however, improved when Spirulina extract was added, and this improvement depended on the concentration employed.

Shelf-life analysis of the quality of the bread using the film

Bread, among other foods, is one of the most culturally, religiously, and socially significant. But, as society has evolved over time, there has been a significant shift in how bread is made, bought, and consumed, necessitating a longer shelf life for this very perishable item. When bread is stored, a variety of physical and chemical changes take place that cause its nutritional value to gradually decline. Staling includes structural alterations, including a rise in crumb stiffness and crumbliness from moisture loss and starch retrogradation, as well as flavour and odour changes from oxidative events. With the use of various packaging techniques, namely those that inhibit oxidation and moulding, bread shelf life can be increased (Pasqualone, 2019). Chitosan has significant potential for use in the food processing industry because of its unique physicochemical qualities, high biodegradability, biocompatibility with human tissues, antibacterial and antifungal activity, and no toxicity (Aider, 2010).

Essential oil-infused films and coatings also excelled pure films and coatings in food systems against postharvest fungi and foodborne bacteria. When compared to pure chitosan films and coatings, the use of EO-containing chitosan films and coatings resulted in an increase in shelf-life and a reduction in lipid peroxidation of fish and meat items (Yuan *et al.,* 2016). In this study, the sample packed in low-density polyethylene (LDPE) served as the control. Visible fungal growth appeared on the surface of the control sample after 5 days of storage at 24 ℃.

Fig 4 Bread packed in the developed edible film

Fig 4.1 Bread packed in LDPE at 10th day

Results from the table 6 show that the preliminary antifungal activity evaluation indicated that the incorporation of lemon seed into pure chitosan film improved the antifungal properties of the film and significantly retarded the proliferation of fungi on bread samples. When bread samples were packaged, synthetic plastic showed fungal growth proliferation in 6 days, while grapeseed extract chitosan film showed the same results at 20 days. (Jha, 2020). Tan *et al.*, 2015 stated that when compared to control samples, wrapping bread samples with chitosan-based grape seed extract composite films prevented fungal growth proliferation. The formulation with glycerol and aloe vera altered the antifungal activity of fresh chitosan films, resulting in a 60% suppression of fungal (*Colletotrichum gloeosporioides*) growth. (Barragán-Menéndez *et al.*, 2020).

Bread preserved with sodium caseinate/glycerol edible film wrappers was substantially softer than unpackaged bread for 6 hours, although they weren't as effective as PVC. The edible films had a low water vapour permeability and strong tensile strength.(Schou *et al.*, 2005). Sliced bread was stored with antimicrobial sachets that contained oregano essential oil to slow the growth of yeast and mould. The release of essential oil altered the sensory qualities of bread because the level of γ-terpinene and ρ-cymene rose in bread during storage compared to control samples. (Passarinho *et al.*, 2014). Essential oils of marjoram (*Origanum majorana*) and clary sage (*Salvia sclarea*) combined into a paper disc dramatically inhibited mould development on bread slices in a petri dish, with marjoram yielding greater effects. The strong odour imparted to bread by essential oil vapours implies that this preserving strategy can be employed until new options are identified; otherwise, the sensory aspects may be too strong (Pasqualone, 2019)

Table 6 Antifungal activity of the control sample (LDPE) and the lemon chitosan film

S. No	Package material	Day 1	Day 5	Day 10
1	LDPE	Good	Some whitish growth is seen on bread surface	Fungal growth seen
2	Lemon chitosan film	Good	Good	Good

As a paper covering, wax paraffin containing cinnamon essential oil had a considerable effect on Rhizopusstolonifer inoculated in bread. (Rodríguez *et al.*, 2008) Nevertheless, (Kechichian *et al.*, 2010)attempts to use a cassava starch film with cinnamon powder for sliced bread packaging were unsuccessful due to the deleterious influence of bread moisture on the physicochemical qualities of the film. Sliced bread kept for 15 days in methylcellulose films with nanoemulsions of clove bud (*S. aromaticum*) and oregano (*Origanum vulgare*) essential oils reduced yeast and mould growth. The films containing the same essential oil's traditional emulsions had reduced antibacterial qualities, suggesting that nanodroplets had a higher bioavailability. Consequently, if preservative is enclosed in nanoparticles, a smaller amount may still be able to provide the same antibacterial effectiveness (Otoni *et al.*, 2014).

CONCLUSION

The edible film was prepared by incorporating lemon seed extract into chitosan. The thickness, mechanical strength, wettability, and swelling properties of the films were affected by the lemon seed extract. The thickness of the chitosan films and elongation at break values were increased, with the addition of lemon seed extract and it had a negative correlation with the tensile strength results. The water contact angle values indicated that the surface hydrophobicity of the developed film decreased with lemon seed extract. Moreover, the developed films tended to decrease oxygen permeability. The antioxidant activity of films enhanced with extract addition shows the potential of films to preserve food that has high sensitivity to oxidation. The lemon seed extract can be evaluated as a low-cost filler to enhance the properties of biopolymer-based packaging films. Results from preliminary antifungal activity evaluation demonstrated that the developed films also have the potential for retarding the incidence of fungal growth and could increase the shelf life of the bread. Further efforts should be realized on the antimicrobial properties and biodegradation behaviour of these films. Considering the evaluated properties, the biowaste-incorporated edible films can find potential applications in the active packaging industry as a partial alternative to synthetic counterparts.

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