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Synergistic Effect of Citric Acid and Ascorbic Acid in Antimicrobial Packaging Films for Extending the Shelf Life of Fresh Produce

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ABSTRACT

The current study aims towards the preparation of chitosan/gelatin based antimicrobial films at different concentrations of citric acid and ascorbic acid (1, 1.5 and 2.0%) to enhance the shelf life of food commodities. The films were made with equal ratios of chitosan and gelatin. Citric acid and ascorbic acid were incorporated (1, 1.5, and 2.0%). The solution was poured on 30 mL petri dishes and dried for 3 days. The films showed a decrease in trend in thickness with increase in concentration. The transparency increased for citric acid films and decreased for ascorbic acid. The viscosity also increased with increasing concentrations. Also, the porosity of films increased except for the film having 2.0% ascorbic acid. Both films showed antimicrobial effect against *S. aureus* but only ascorbic acid showed inhibition against *E. coli* as well. This indicates that citric acid films are not appropriate for foods affected by *E. coli*. Although, significant changes were seen in the results. The films having 1.5 and 2.0% citric acid was highly effective against *S. aureus* with an inhibition zone of 13.35±0.18 mm. Furthermore, ascorbic acid showed highest antimicrobial activity against *S. aureus* at 2.0% and against *E. coli*, the film having 1% ascorbic acid was highly effective. The moisture barrier properties also showed significant changes with an increase in concentrations. Films having 1.5 and 2.0% citric acid exhibited higher efficacy against *S. aureus* but not against *E. coli.* Also, the films having 2.0% ascorbic acid can be utilized against *S. aureus* and 1.0% ascorbic acid films can be effective against *E. coli*. Foods that have the tendency to be affected by *S. aureus* can be packed using 1.5 and 2.0% citric acid films but their moisture barrier property is not good. Similarly, 1.0% ascorbic acid films can be used for foods that can be affected by *E. coli*.

INTRODUCTION

Packaging imparts a great influence over preservation, dispersal and marketing of foodstuffs. With the increase in consumer demand and variation in market trends, food-active packaging studies are progressing greatly. Recently, researchers are shifting their focus on the creation of edible films produced from natural sources as they are highly biodegradable, biocompatible and edible. They also have other practicable applications. The films carry several active ingredients that may include preservative compounds (antimicrobial/antioxidant agents), flavors, spices and colorants, *respectively*. Antimicrobial packaging is a crucial part of active packaging systems that has aspired to prolong the shelf life of food commodities and ensure that the foods are secure and of higher quality (Tripathi *et al.*, 2025). Packaging materials (those intended for food) must meet a certain number of requirements such as effective conservation of the product against harmful factors (e.g., oxygen, UV radiation, moisture, bacteria and fungi) during food processing, transportation and storage. In this regard, the materials possessing the traits of active packaging are becoming more popular. The major benefit of using food packaging materials with antibacterial properties is extending the shelf life of foods and protect various products from bacteria and fungi which can be dangerous to human health (Barlow and Morgan, 2013; Cardoso *et al.*, 2017; Al-Tayyar *et al.*, 2020; Sharma *et al.*, 2020). These antimicrobial films inhibit the microorganisms from growing on the fruit's exterior, minimizing risk of spoilage and prolonging the shelf life. These films and coating materials create a barrier that prevents the attachment and growth of harmful bacteria, making the fruit safer for consumption. Furthermore, they preserve the nutritional quality of fruits by slowing down the deterioration process (Lim *et al*., 2012).

Chitosan is produced from chitin, the most abundant polymer followed by cellulose. It is possible to improve the mechanical, thermal, barrier, antibacterial and antioxidant qualities of chitosan-based films (Mujtaba *et al*., 2019). With the help of chitosan researchers have created thin and protective films and applied on the surface of fruits or other materials. It is a natural biopolymer extracted from crustacean shells, making it biodegradable, eco-friendly and co-existing with sustainable practices. Chitosan has innated antimicrobial properties (Imawan *et al*., 2022).

Gelatin is a protein that is appropriate for food packaging because it is renewable, biodegradable, low cost, can form films and are edible (Esteghla *et al*., 2018; Mujtaba *et al*., 2019). Gelatin is a protein that can easily solubilize in water and is yielded from partially hydrolizing the collagen, which is majorly used in the food and medicinal industry. Gelatin films are transparent biodegradable and have low oxygen permeability (Wang *et al*., 2022). However, they exude ineffective shielding effect against moisture and under high relative humidity, they show intermediate mechanical strength (Nemes *et al*., 2020).

Moreover, gelatin also possesses innate antimicrobial properties, making it convenient for developing antimicrobial films. These films can help to slow down the growth of microorganisms. Further, citric acid, also known as the tricarboxylic acid ($C_6H_8O_7$) is a preservative that can act as an acidulate, a flavoring compound, an emulsifier, a sequestering agent and a buffering compound that is widely used across many industries, mainly food industry. It is mildly hygroscopic and has high water solubility (62.07% at 25 °C). It has antioxidant and antimicrobial properties against food-related pathogens including *Escherichia coli*, *Salmonella, Listeria monocytogens* and prevents spoilage. Citric acid also acts as a natural preservative, expanding the storage life of food products and hindering microbial contamination when incorporated into antimicrobial films (Burel *et al*., 2021). The citric acid (CA) also has the ability to serve as multifunctional cross-linker through hydrogen bonding with carboxymethyl chitosan (CMCS), it also worked as effective reinforces to enhance mechanical and antimicrobial efficacy of composite films (Wen *et al*., 2021).

Ascorbic acid (AA) is found in nature and possesses high polarity and efficient antioxidant activity. Ascorbic acid is typically found in several foods that include mango, orange, lemon, blackberry and vegetables (Janani *et al*., 2020).

MATERIALS AND METHODS

Procurement of Raw Materials

Analytical grade tween-20, glacial acetic acid, glycerol, chitosan was purchased from Sigma Aldritch St. Louis Missouri USA and Merck Germany. Extra pure grade citric acid and ascorbic acid were procured from (Seohaean-ro, Siheungi-si, Gyeonggi-do, Korea). Moreover, gelatin was procured from local market of Karachi.

Preparation of Antimicrobial Film

Antimicrobial films and coatings were developed by using different proportions of citric acid and ascorbic acid. In a beaker 90 mL distilled water was added followed by the addition of 1 mL glacial acetic acid. The solution was stirred using a magnetic stirrer. After that 1 g chitosan was dissolved at an increased temperature (45-50 °C) for 15-20 mins. Then 1 gm gelatin was added, keeping the temperature at 65 to 90 °C for 5 mins. Furthermore, glycerol (1%) was added as a plasticizer, tween 20 (1%) as an emulsifier and citric acid and ascorbic acid (1, 1.5 and 2%) as an antimicrobial agent to the solution. The mixture was stirred through stirrer for the preparation of a homogeneous solution. For the preparation of film casting method was utilized. The solution was poured on a 30 mL petri dish or plate and then dried at 40-45 °C for 24 hr. for thickness, proper consistency & mechanical property. The main ingredients and antimicrobial ingredients were added and then plasticizer & emulsifier for the development of mechanical property. After homogenized mixing this solution was transferred into a petri plate and placed in hot air oven for 24 hr. Less viscous film takes less time as compare to thick film.

Characterization of Antimicrobial Films and Coatings

Antimicrobial films and coatings were characterized for physiochemical (thickness, transparency and particle size), rheological (viscosity), moisture barrier properties (water vapor permeability) and antimicrobial effect of the film (inhibition zone).

Thickness

A digital micrometer was used to measure the thickness of the film at ten randomly chosen points with a precision of 0.001 mm. The water vapour permeability and mechanical characteristics were ascertained using the average thickness that was found from these experiments. To calculate the tensile and piercing strengths, the film thickness was also measured using a micrometer to the nearest 0.02 and 0.04 mm. Whereas the mean for piercing tests was determined by taking ten random measurements on each film, the mean for tensile testing was determined by averaging two measurements conducted on each film specimen. Additionally, the inhibitory efficiency of the film at various thicknesses was assessed using the film's thickness (Hosseini *et al*., 2015).

Viscosity

The viscosity of the film was measured using a rheometer in compliance with a specific protocol developed by Jouki *et al*. (2014). The concentric cylinder probe was left to adjust at 25°C for ten minutes following the addition of the 30 mL sample. After that, the sample was sheared at a constant temperature of 25°C for a period of 1 to 180 seconds.

Optical properties

The transmittance of the antimicrobial coatings was investigated across a 600 nm wavelength range using the UV-Vis absorption spectrometer. Each film sample, measuring 10×40 mm^2, was put into a quartz cuvette with a 1 cm channel length, whereas a control cuvette was an empty cuvette. The UV absorption of the film samples was calculated by subtracting the UV absorption of the empty cuvette. This transmittance measuring process was done three times for each sample.

The transmittance was calculated using the following formula based on A = − lg (T);

$$T = \frac{1}{10A}$$

Here,
A= absorbance
T = transmittance of film
Samples were measured at a particular wavelength. The samples opacity was calculated using the prescribed procedure by Cazon *et al.* (2017) as they used the following equation:

$$\text{Opacity} = \frac{A_{600}\ \text{nm}}{d}$$

Where A_{600} nm is the absorbance at 600 nm and d (mm) is the thickness of films.

Moisture Barrier Properties

To evaluate the moisture barrier properties of antimicrobial films, the water vapour permeability of the film samples was measured using the ASTM official method, which was also utilized by Brzoska *et al.* (2018). The films were carefully affixed to Payne permeability cups, which were maintained at a temperature of 25 °C and a relative humidity of 65%. Each cup held 10 mL of distilled water. Weight fluctuations were recorded every hour for ten hrs. The water vapour permeability of the films was calculated using the following formula:

$$WVP = \frac{\text{Slope} \times L}{A \times \Delta P}$$

Where;
Slope= Constant representing weight change vs time calculated via linear regression
L= Average film thickness (m)
ΔP = Partial water vapor pressure difference
A= Moisture transfer area of cup (m^2)

Antimicrobial Effect of the Films

The precise bacterial cells that were targeted were chosen. The Guo *et al.* (2020) technique was somewhat changed in order to revive and cultivate freeze-dried cells. One colony was given Nutrient Broth (NB) and it was then cultured in an air bath incubator with a reciprocal shaker set to 37 °C for 17 hrs. at 180 rpm. Following a 10-minute centrifugation at 4000 rpm and 4 °C, the cells were cultured twice in 0.1% peptone monohydrate. Initially, it was discovered that the solution contained 109 logs CFU/mL of *E. coli* and *S. aureus* cells. Next, in order to reduce the final concentration of *E. coli* and *S. aureus* cells to 104 logs CFU/mL, 0.1% peptone water was added to this suspension. The antibacterial activity of many bionanocomposite films against *S. aureus* and *E. coli* was assessed in peptone water using the recommended protocol. With respect to the previously outlined procedure, 16 × 150 mm^2 culture tubes were filled with 10 mL of E. coli suspension after 1.5 x 2.0 cm^2 films had been cut to size. Tubes without any film samples were used as controls. Following incubation times of 0, 6, 12 and 24 hours, bacterial suspensions were serially diluted using 0.85% sterile saline solution with the assistance of a reciprocal shaker set at 22 °C and 100 rpm. On PCA plates, 100 μL of the suitable dilutions were disseminated to identify the *E. coli* and *S. aureus* surviving populations. Incubation of these plates was done at 37 °C for 24 hr. later the bacterial colonies were quantified.

Antimicrobial Activity

Bacterial cultures

E. coli and *S. aureus,* obtained from the Microbiology Research Lab in Karachi, were used as the test pathogens. The remaining strains were kept at -18 °C in glycerol. *E. coli* and *S. aureus* were sub-cultured into nutritional broth for a full day prior to testing.

Agar well diffusion test

S. Aureus and *E. coli* species were diluted to obtain 10^4 CFU/mL concentrations. Following this, 8 mm diameter wells were made in the agar medium using 100 µL of the bacterial dilution that had been spread onto standard plate count agar. Next, a combination or solution containing ascorbic acid and citric acid at different percentages (1.0, 1.5 and 2.0%) was added to these wells in a volume of 25 µL. As the control, a well holding 25 µL of an antimicrobial filmogenic solution was chosen. The petri plates were then incubated at 37 °C for a whole day. In order to assess the antibacterial activity, the diameter of the inhibition zone, which encompasses the diameter of the well, was measured using the technique by Samar *et al.* (2022).

Porosity

The insoluble solvent approach developed by Liang *et al.* (2016) was used to calculate the porosity of the film. The films were, in essence, saturated with chloroform and the weight of the film samples was noted both prior to and following immersion. After that, porosity was determined using a specific formula.

$$\text{Porosity} = \frac{(m_2 - m_1)}{\rho V} \times 100$$

Where m_1 and m_2 are the weight of the film before and after immersion in the chloroform, respectively. V is the volume of the film before immersion, which was calculated using formula, length × width × height of the film, and ρ is the density of chloroform.

Statistical Analysis

The data collected for each parameter was passed through the accurate statistical design (Montgomery, 2017). Statistical software (Statistix-8.1) was used to conduct analysis of variance (ANOVA) to determine the level of significance ($P<0.01$ & $P>0.05$) for transparency, thickness, viscosity, porosity, moisture barrier property and inhibition zone of antimicrobial films. Furthermore, comparison of means was done by Tukey's Honest Significant Difference.

RESULTS AND DISCUSSION

Transparency

Table 1 indicates a non-significant trend for transparency among the treatments for citric acid and ascorbic acid films. The highest transparency was obtained at 1.5%

citric acid and 1.0% ascorbic acid. Usually, transparency is expected to be higher than 75% that means our values are in accordance to the accepted range. Transparency can be affected by the type of plasticizer and stabilizer and particle size. When light absorption initiates chemical processes in dietary ingredients such as proteins, lipids, pigments and vitamins, the process is called photodegradation. The loss of vitamins is the main effect on nutritional value. Moreover, photodegradation imparts a strange tastes and color to packed foods. According to the study, light absorbance is directly related to the opacity of the films. In order to prevent photodegradation impacts on food goods, it is important to know these processes in order to preserve food quality and nutritious content. This creates a need for packing materials that minimize light exposure (Han and Hanani, 2020). Additionally, they claim that film opacity, a crucial component of food packaging, reveals the film's transparency degree. High-transparency films are favored because they make it simple to monitor the state and appearance of the food while it is being stored. Although opaque films shouldn't be used for packing in general, they might be okay for goods that are light-sensitive (Han *and* Hanani, 2020).

Priyadarshi *et al*. (2018) prepared glycerol plasticized modified chitosan films incorporated with citric acid. They also noticed that after modification the films had a lower opacity and increased transparency which was 81%. Therefore, our values are compatible with this value.

Thickness

The Table 1 shows that the thickness of antimicrobial films showed significant changes among treatments. Thickness decreased significantly as the concentration of the citric acid films and ascorbic acid films increased in the treatments. The film having 1% citric acid had the highest thickness among other treatments. The film having 1.5% ascorbic acid showed the lowest thickness. The mechanical strength of the film as well as its capacity to function as a barrier against gases and moisture are significantly influenced by its thickness. The films can't properly stop gases and moisture from penetrating if they are too thin. On the other hand, excessively thick coatings can impede the process of respiration, which could result in anaerobic circumstances and cause fruits and vegetables to produce alcohol, which could provide unwanted off flavours. Antimicrobial films showed a significant drop in thickness as particle size increased, most likely as a result of moisture loss during the drying process. According to Akhtar *et al.* (2022), the use of plasticizers and emulsifiers, which give the films a flexible and smooth structure, also affects the thickness of the films. A related study by Wu *et al*. (2019) also demonstrated an increase in film thickness and density when compared to the PS/CS film control with additional citric acid. Films with higher citric acid content had a more pronounced effect ($p < 0.05$). This suggests that the concentration of particles in the formulation and film thickness are clearly correlated. Another study by Rodríguez *et al*. (2020) also indicated that the edible papaya films showed a range of thicknesses; 0.13±0.04 mm for films dried in a dehydrated environment and 0.32±0.03 mm for films dried at ambient temperature. The thickness of the oven-dried films was 0.26±0.01 mm, which is an intermediate value. These values are also similar to our values. They also stated that films are dried at room temperature may have higher thickness caused by slower and less controlled drying process at this condition as compared to the others tested films.

Viscosity

The Table 1 shows a significant trend in the viscosity of the antimicrobial films among treatments. The viscosity of the films showed improvement substantially as the concentration of the citric acid and ascorbic acid in films increases. The highest viscosity was obtained for the film having 2.0% ascorbic acid while the lowest viscosity was obtained for the film having 1.0% citric acid. The average range for both films coincides with similar present studies. According to Akhtar *et al*. (2022), the reason behind a higher viscosity could be the development of a jelly-like structure is made feasible by the presence of biopolymers. To obtain the appropriate film thickness, increased viscosity is required for optimal film formation. Films with low viscosity lose their moisture quickly during drying and cannot obtain proper thickness. The viscosity of the different treatments showed a linear increase as the concentration of citric acid and ascorbic acid increased that might be due to larger particle size formation in the films which caused resistance in the flow. The reason behind this could be the high ionic strength of these solutions. Also, the viscosity of polymer solutions might be related to concentration, molecular weight, temperature and ionic strength. Assuming such correlation exists, it is difficult to examine chitosan film properties from solutions. However, they can be predicted in more concentrated forms when the gelation of macromolecular solution occurs. Akyuz *et al*. (2017) states that the source of chitosan has an influence on the deacetylation degree, molecular weights (viscosities of chitosan solutions) and also functional properties of chitosan solutions and films. Further, study done by Mujtaba *et al*. (2018) indicates that acid-dissolved chitosan films have a wide range of beneficial physicochemical and biological characteristics. These characteristics show a great deal of variation, depending on variables including the type of composite, percentage of acid dissolution, drying temperature, solution viscosity and degree of acetylation. The film's remarkable adaptability in composite formulations comes from its easy incorporation with other materials.

Porosity

According to Table 1, significant change can be observed in the porosity of the antimicrobial films among treatments. The porosity of the films had a substantial change as the concentration of the citric acid and ascorbic acid in films increases except for the film having 2.0% ascorbic acid. The highest porosity for films having citric acid was obtained at concentration of 2.0%. As for the films having ascorbic acid, the highest porosity was obtained at concentration of 1.5%. Citric acid has the ability to form multiple salt bridges with amino groups which indicates that it acts as a reticulating agent that contribute in gel formation and the film porosity. However, ascorbic acid is majorly used as an antioxidant in packaging films. A similar pattern was seen in the research conducted by Singh *et al*. (2021). They stated that the mechanical strength of the packaging film is impacted by the movement of active components, which is mostly controlled by the porosity and integrity of the film. Porosity in the test and control films ranged from 0.12 to 0.34%. The results showed that as compared to the control film, the test films were noticeably more porous. High viscosity is also known to have an impact on elongation at break, water absorption and water vapor permeability (WVP). Chen *et al.* (2011) obtained chitosan membranes using freeze-gelation method. The membranes had a homogenous interconnected pore structure. The porosity of the membranes obtained was 0.9 meaning that 90% of the membrane was occupied by pores. They also found that there was so significant relation between acid solutions and porosity of the membranes. Hence, it is possible that the concentration of acids in our films had no effect on their porosity.

Table 1
Mean value for transparency, Thickness, Viscosity and Porosity of antimicrobial films

Treatments	Transparency	Thickness	Viscosity	Porosity
T_0	95.04±3.10^a	0.38±0.01^a	0.12±0.01^c	11.95±0.04^e
T_1	96.56±3.10^a	0.36±0.03^{ab}	0.13±0.02^{bc}	15.31±0.04^e
T_2	96.51±3.10^a	0.34±0.021^b	0.15±0.02^{ab}	77.42±2.36^d
T_3	96.40±2.93^a	0.27±0.041^c	0.13±0.01^{bc}	123.50±4.08^b
T_4	91.35±2.93^a	0.19±0.051^d	0.14±0.02^{abc}	148.90±4.08^a
T_5	94.88±3.49^a	0.26±0.021^c	0.16±0.01^a	108.39±4.08^c

Figure 1
Transparency (%) of antimicrobial films

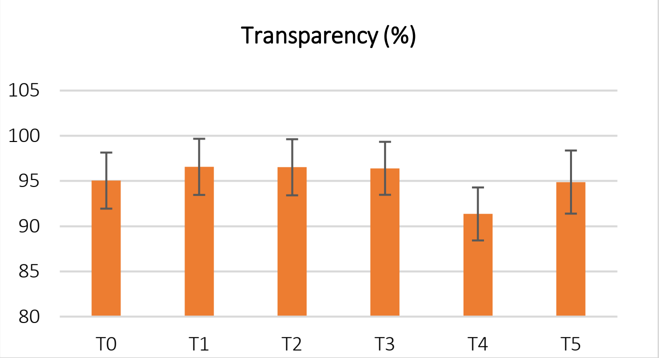
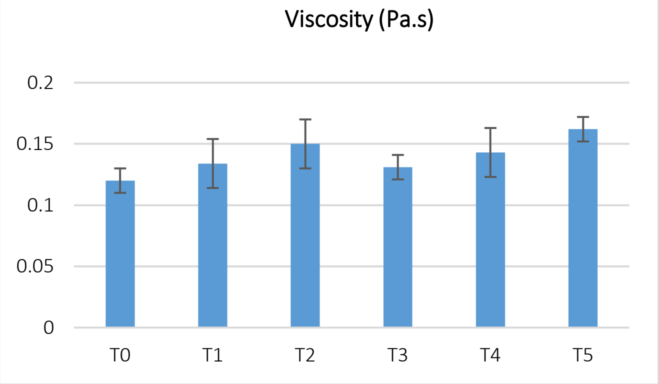


Figure 2
Viscosity (Pa.s) of antimicrobial films



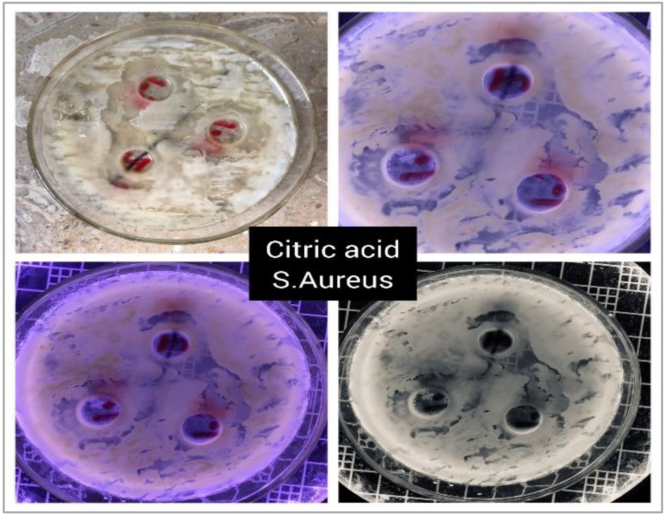
Antimicrobial Activity

The antimicrobial activities of the citric acid and ascorbic acid films was observed against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) food-borne pathogenic bacteria as shown in the Table 2. Predictably, the citric acid films did not show any antimicrobial activity against *E. coli*, while ascorbic acid exhibited antimicrobial effect against both of the test organisms. It was observed that the effect of film having 1.5 and 2.0% citric acid was more enhanced than film having 1% citric acid. In case of ascorbic acid, the film having 2.0% ascorbic acid was highly effective against *S. aureus* while film having 1.0% ascorbic acid was highly effective against *E. coli.* According to research, binding to the bacterial cell membrane of organic acid antibacterial agents disrupts the production system between proteins and the cell membrane, which in turn stops bacterial growth. This is the main antimicrobial mechanism of organic acid antibacterial agents according to Zhijun *et al.* (2017). They conducted a similar study to check the effect of Starch/Polyvinyl Alcohol/Citric Acid ternary blend on Gram-positive bacteria (*L. monocytogenes*) and Gram-negative bacteria (*E. coli*). They discovered that *L. monocytogenes* was more sensitive to films containing citric acid than *E. coli*. The films have antibacterial qualities thanks to citric acid, which is well known for its acidic qualities. This is a trait that is frequently apparent. Additionally, Chang *et al.* (2021) looked at the Gram-positive and Gram-negative bacteria, *Staphylococcus aureus* and *Escherichia coli*, correspondingly. They assessed the antibacterial efficiency of chitosan film samples using the methodology described by Riaz *et al.* (2020). Previous studies indicate that the presence of active chemicals with UV–vis absorption characteristics is what gives some antimicrobial chitosan films their anti-UV barrier qualities.

Table 2
Mean for inhibition zone (mm) of antimicrobial films

Treatments	Means
T_o	12.80±0.03^b
T_1	13.35±0.18^a
T_2	13.35±0.2^a
T_3	10.00±0.04^d
T_4	12.00±0.02^c
T_5	13.20±0.2^a
T_6	9.60±0.1^e
T_7	9.20±0.03^f
T_8	8.00±0.08^g

Figure 3
Antimicrobial activities of the Citric acid and Ascorbic acid films against Streptococcus aureus



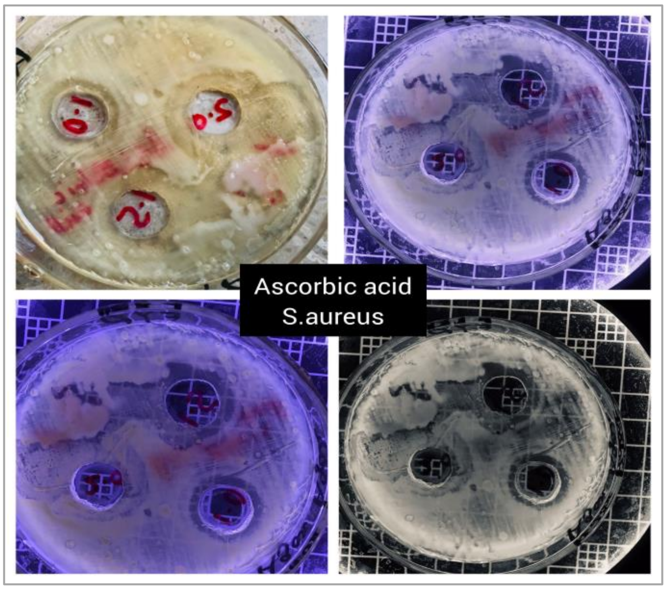
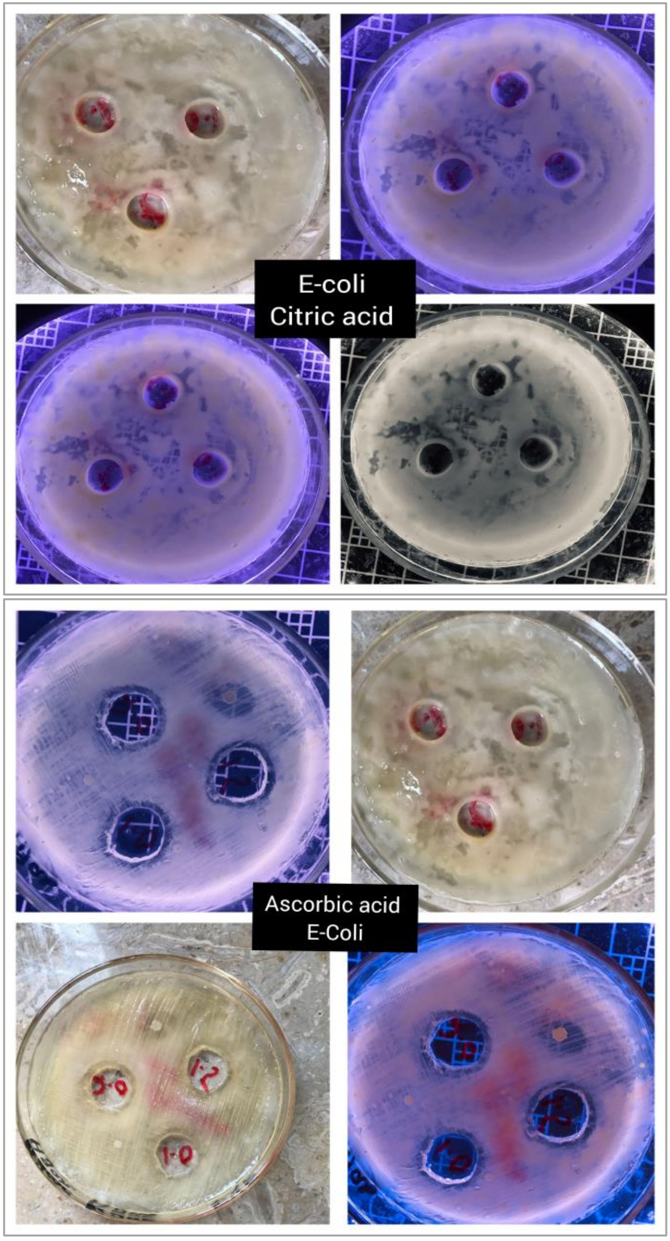


Figure 4

Antimicrobial activities of the Citric acid and Ascorbic acid films against E. coli



Moisture Barrier Property

According to Table 3, all the films showed a significant change in their moisture barrier properties among the treatments. The highest value obtained for 1% citric acid film. As for ascorbic acid films, the highest value was obtained at 2.0% concentration. At 1.5% citric acid the film exhibited the lowest moisture barrier property and 1.5% ascorbic acid film also exhibited the lowest moisture barrier property. These values are similar to the ones present in recent studies. The reason for fluctuation in readings could be due to irregular humidity and storage conditions. Moisture barrier property is affected by several factors like particle size, porosity and thickness of the film. It is also affected by the plasticizer and stabilizer used in the films. If the films have large pore size then evaporation is more likely to happen rapidly. Butt *et al.* (2023) states that films that retain moisture content or exhibit higher moisture content can lose their flexibility and may degrade following a microbial attack. But this can be counteracted by adding antimicrobial agents in the films. The ability to keep out moisture is crucial for food packing. The degree of moisture that permeates the packing material significantly impacts the quality of the food packaging. In dry food packaging, a moisture barrier is required to prevent external moisture from entering the food container and compromising food quality. In contrast, the barrier in the packaging of fresh produce prevents moisture from escaping the meal, hence averting dehydration as stated by Priyadarshi *et al.* (2018). They assessed the water vapour permeability (WVP) and transmission rate (WVTR) of glycerol-plasticized modified chitosan films (CSCG) containing citric acid in contrast to chitosan films (CS). The modified chitosan films with glycerol plasticization had WVTR and WVP values that were 5.5 and 29% lower, respectively, then the chitosan films. The values match the range that we have.

Table 3

Mean for moisture barrier property (g.mm/kPa.h.m^2) of antimicrobial film

Treatments	Means
T_0	8.73 ± 0.01^{a}
T_1	1.27 ± 0.03^{d}
T_2	1.48 ± 0.04^{c}
T_3	1.21 ± 0.03^{e}
T_4	1.15 ± 0.02^{f}
T_5	1.52 ± 0.02^{b}

CONCLUSION

The thickness decreased with the increase in concentrations of the films. The highest thickness was obtained at 1% citric acid (0.38±0.01 mm) and 1% ascorbic acid (0.27±0.03 mm). The transparency was observed above 75%. The films viscosity increased with increasing concentrations. The highest viscosity was obtained at 2.0% citric acid (0.15±0.02 Pa.s) and 2.0% ascorbic acid (0.16±0.01 Pa.s). The porosity of the films showed a significant change with the increase in concentration. The porosity of the films had a substantial change as the concentration of the citric acid and ascorbic acid in films increases except for the film having 2.0% ascorbic acid. Both films showed antimicrobial activity against *E. coli* and *S. aureus.* The films having 1.5 and 2.0%

citric acid was highly effective against *S. aureus* with an inhibition zone of 13.35±0.18 mm. Furthermore, ascorbic acid showed highest antimicrobial activity against *S. aureus* at 2.0% against *E. coli*, the film having 1% ascorbic acid was highly effective. Films having 1.5 and 2.0% citric acid exhibited higher efficacy against *S. aureus* but not against *E. coli*. The moisture barrier properties of both films showed a significant change. It decreased with the increase in concentrations. The highest value was obtained at 1% citric acid (8.73±0.01) and at 2.0% ascorbic acid (1.52±0.04). The best results were obtained at 1% citric acid and 2.0% ascorbic acid. The films can be used antimicrobial packaging materials for different commodities (mainly fruits and vegetables). Further analysis can be done through SEM, FTIR and UTM for determining the strength and internal structure of our films. A shelf-life study might help up determine their effects for a specific period of time and the appropriate storage condition.

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