

## Original Research Article

# Fabrication and Investigation of Gondhoraj (*Citrus hystrix*) Zest Edible Wrap for enhanced quality and better shelf-life of Chicken Breast

### ABSTRACT:

**Aims:** The inclusion of zest of Gondhoraj (*Citrus hystrix*) essential oil (GPO) in active packaging may provide safety and prolong the shelf life of chicken meat. Therefore, the goals of the study were to assess the physicochemical, antioxidant, and antibacterial properties of GPO added to a starch-based edible film (GF) designed for the packaging of chicken breast meat.

**Study Design:** The study has designed to formulate an active packaging film with the addition of GPO at concentrations of 0.2%, 0.4%, and 0.8% (v/v) in pre film forming starch solution.

**Place of the study:** Department of Food Technology, Guru Nanak Institute of Technology, Kolkata

**Methodology:** The films were made using the casting technique, and their opacity and light barrier were measured by exposing them to light absorption between 200 and 800 nm. The film's moisture content, antibacterial and antioxidant properties, were all assessed. The coliform count for chicken meat packed with GF was then evaluated.

**Results:** The results demonstrate that GF has a lower water solubility ( $0.068 \pm 0.09\%$  -  $0.090 \pm 0.00\%$ ) in comparison with control starch film and a greater opacity value ( $3.150 \pm 0.03$  -  $8.924 \pm 0.09$ ) with respect to light absorption between 200 and 800 nm. Antioxidant activity was demonstrated by diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) at  $70.36 \pm 2.48\%$  and  $83.78 \pm 0.54\%$ , respectively. After being refrigerated at  $4^{\circ}\text{C} \pm 1$  hours, the chicken sample GF had the lowest coliform level ( $4.88 \pm 0.06$  CFU/g) compared to uncoated chicken meat sample.

**Conclusion:** The shelf life study of both coated and uncoated the chicken samples revealed no significant difference ( $p < 0.05$ ), leading to the conclusion that the organoleptic qualities of the chicken are not influenced by GPO. Consequently, the GF exhibited strong antibacterial and antioxidant

characteristics of the film that may help to enhance the nutritional quality as well as prolong the quality of chicken meat over an extended period of time during refrigeration.

**Keywords:** *Antibacterial properties, Chicken Breast, Edible Packaging, Gondhoraj Peel , Shelf-life*

UNDER PEER REVIEW

## 1. INTRODUCTION:

The crucial function that essential oils (EOs) play in food as natural preservatives has led to intensive research on their antimicrobial and antioxidant properties. EOs are the bioactive ingredient found in active packaging, such as antimicrobial packaging, and they have greater efficacy in reducing pathogens in perishable goods [1]. The main components of the steam-distilled- *Citrus hystrix* oils from Selangor, Malaysia were limonene (19.8%), citronellal (7.8%),  $\beta$ -pinene (16.8%), sabinene (35.2%), and  $\alpha$ -pinene (3.1%). However, according to study, the compounds that were gathered from northwest Thailand included limonene (34.32%),  $\beta$ -pinene (17.4%), terpinen-4-ol (10.20%),  $\alpha$ -terpineol (8.76%),  $\alpha$ -pinene (3.59%), and sabinene (1.59%) [2]. Compared to Gondhoraj leaf oil and twig oil, fresh fruit peel contains the highest levels of antioxidant and antibacterial activity. This is because, in comparison to leaf and twig oils, fruit peel oil has a larger concentration of monoterpene hydrocarbons (pinene, sabinene, myrcene, and limonene) [3]. The highest DPPH antioxidant and antibacterial activity against *Escherichia coli* is actually seen in fruit peel oil, which has a far lower citronellal concentration than leaf oil [4]. One of the newest applications of antimicrobial chemicals integrated in packaging materials to inhibit bacteria development is active packaging [5]. Various studies have shown that incorporating Citrus rinds as powder or as EOs into food products may enhance the food's quality without negatively affecting the sensory attributes when added at the right amount.

According to prior studies, starch is a great example of a polysaccharide that can be used because of its ability to build an oxygen-permeability-low, colourless, and odourless polymer matrix [6]. According starch has the capacity to build a colorless, odorless polymer matrix with minimal oxygen permeability, hence it is useful [7]. The starch-based film exhibits favorable characteristics such as homogeneity, flexibility, transparency, and rapid biodegradability [8]. In biodegradable film production, starch is a viable raw material because it may be utilized without any preliminary processing and with or without the inclusion of plasticizers, and can be obtained with ease employing the casing method, serving as active ingredient carriers [9]. Given the importance of packaging for sustainable food consumption, the world's plastic production rose by 4.2% between 2015 and 2016 to 335 million tons [10]. However, a significant portion of plastics are non-biodegradable and persist in the environment for a long time [11]. In order to solve this issue and provide the best packaging solution to help reduce food losses and the accumulation of plastic, ingenious sustainable packaging is crucial [10].

According to statistics, the amount of chicken meat consumed worldwide in 2017 was 110 million tonnes [12]. Over the next ten years, it is anticipated that the production of poultry meat will reach a target of 134.5 million tonnes [13]. The two main variables that affect the quality of chicken meat are lipid oxidation and microbiological contamination. Cross-contamination is the process through which food-borne viruses spread from the surface to the other portions of the chicken flesh [14]. As a result, concerns about the safety and quality of chicken meat are increasing throughout the whole supply chain. The development of bio-based active packaging with antimicrobial compounds has the potential to reduce adverse environmental impacts and provide protection against food microbiological contamination [15].

It was suggested that plant essential oils could serve as natural alternatives to improve safety and extend the shelf life of poultry meat. The Gondhoraj lime is utilized in South Asian cuisines to enhance the flavour of traditional meals. There are numerous uses for gondhoraj and its peel, including flavoring cuisine, preserving food, and traditional medicine. Additionally, Australia, Brazil, Hong Kong, Thailand, and the United States of America use it as an ornamental plant [16]. However, there is still not much data in the literature about the application of Gondhoraj Peel essential oil (GPO) in food matrices despite its bioactivity. Therefore, the primary objectives of the study were to assess the physicochemical, antioxidant, and antibacterial characteristics of GPO added to a starch-based edible film (GF) for packaging chicken meat during refrigeration.

## **2. MATERIALS AND METHODS**

### **GPO Infused Starch Based Active Packaging**

#### **2.1. Extraction of Peel Oil from Gondhoraj**

As stated in the earlier work, the GPO integrated into the starch-based edible film was extracted using hydro distillation method [17]. With a commercial slicer (Tupperware MandoChef, Florida, US), Gondhoraj Peel (GP) was cut into small pieces of around 0.5–1 cm. To attain +10% moisture content, the GP was lyophilized using a freeze-dryer (VirTis Genesis 25 Pilot Lyophilizer, New York, US) at  $-50^{\circ}\text{C}$  for 96 hours. For additional extraction, dried sliced TGI was ground into a powder using a grinder (Rong Tsong Precision Technology, Taichung, Taiwan) and sieved through a mesh with a diameter of 1000  $\mu\text{m}$ . Approximately 200 g/run of TGI powder were stored in a filter for the hydro distillation process in a lab scale. The essential oils isolated were collected in 2 ml Eppendorff (EP)

tubes, dehydrated over anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ). Thereafter, the essential oils were taken in fresh EP tubes and preserved with proper sealing.

## 2.2 Formulation of The Edible Casing or The Film Forming Solution (FFS)

The films were made by casting a solution that was slightly altered from an earlier process (Medina Jaramillo et al., 2015). In order to make the film-forming solution (FFS), distilled water was used to dissolve 3% (w/v) starch powder (Chemiz, Selangor, Malaysia). The starch was then heated to  $90^\circ\text{C}$  for 30 minutes in order to ensure gelatinization. As a plasticizer, glycerol was added at a concentration of 0.3% (w/v) in relation to the starch. The highest additive doses needed to produce a homogenous FFS were found through preliminary testing. Carrageenan, an emulsifier, at 25% (0.2% v/v of EO) to ensure the uniform distribution of oil throughout the FFS. Following the addition of GPO at concentrations of 0.2%, 0.4%, and 0.8% (v/v), all ingredients were combined and homogenised at room temperature ( $27^\circ\text{C}$ ) using an IKA Ultra-Turrax T8 homogenizer (IKA, Königswinter, Germany) running at 20,000 rpm for two minutes. The FFS was constantly stirred to eliminate air bubbles. A 14 x 14  $\text{cm}^2$  polycarbonate Petri dish plate with a 40 mL volume of the FFS was spread out and let evaporate for 48 hours at  $27^\circ\text{C}$  and  $45 \pm 5\%$  relative humidity (RH) in the dark in tray dryer as shown in fig 1. A similar control film was made without GPO. Prior to testing, the films were kept in a humidity room at  $45 \pm 5\%$  RH and  $25 \pm 2^\circ\text{C}$ .

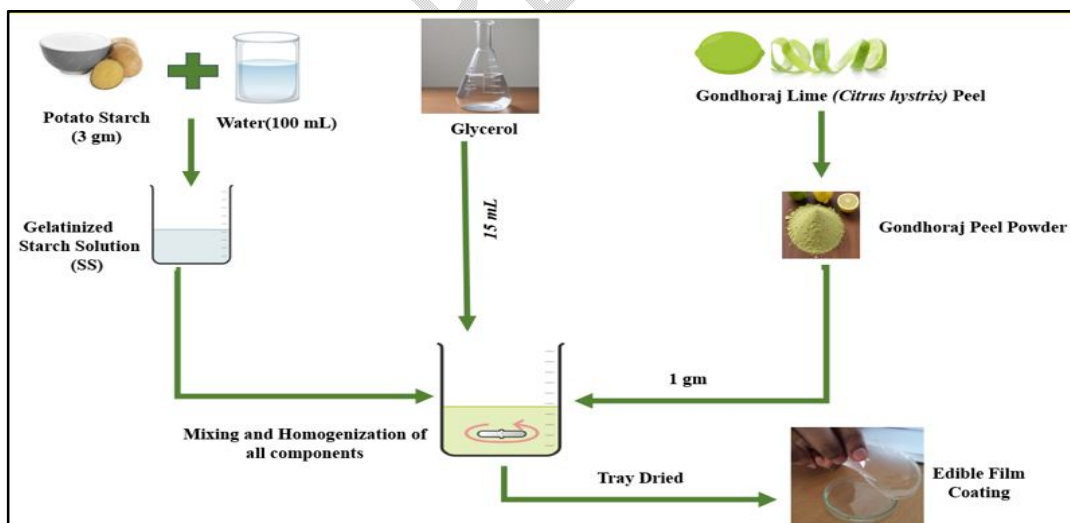


Fig 1: Formulation of The Edible Casing or The Film Forming Solution (FFS)

## 2.3. Physical Characteristics of the Film

### 2.3.1. Thickness of film

The average film thicknesses of the preconditioned samples were determined at 10 random locations on the film using a hand-held digital micrometre (Mitutoyo Co., Kawasaki, Japan) with a sensitivity of 0.001 mm [18]

### **2.3.2. Mechanical Properties**

According to Iwata et al. (2000), the mechanical properties were tested using an Instron 4302 Series IX Machine (Instron Co., Massachusetts, USA). This machine allowed for the measurement of Young's modulus (MPa), tensile strength (MPa), and elongation at break (%). Samples of preconditioned film were divided into 2x9 cm<sup>2</sup> strips. Every strip was evaluated at a crosshead speed of 30 mm/min after being clamped at the top and bottom with an initial grip spacing of 30 mm [18].

### **2.3.3 Transmission of Light and Opacity**

The films' light barrier characteristics were evaluated through the use of a Genesys 10 UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA) to measure light absorption at wavelengths ranging from 200 to 800 nm [19]. After being cut to 1x4 cm<sup>2</sup>, preconditioned film strips were put into a UV cuvette and the spectrophotometer was used to measure the absorbance values directly. Equation 1 was used to determine the films' opacity and light transmission (%) [20]

$$\text{Opacity} = \frac{A_{600}}{x} \quad (1)$$

where x = average film thickness (mm) and A = absorbance value of the film at a wavelength of 600 nm.

### **2.3.4. Solubility of Films in Water**

The film's water solubility has been evaluated using the method [21]. After being sliced into 4x4 cm<sup>2</sup> pieces, the films were oven-dried for 24 hours at 100°C. Before the dried samples were immersed in 50 mL of distilled water in a beaker for an entire day, the initial dry mass was noted. Following the soaking period, the films underwent another 24-hour oven drying at 100°C, and the final dry mass was calculated by Equation 2. [19].

$$\text{Solubility (\%)} = \frac{W_i - W_f}{w_f} \quad (2)$$

Where W<sub>i</sub> = initial dry weight and W<sub>f</sub> = final dry weight.

### **2.3.5. Antioxidant Properties of Film**

### **2.3.5.1. DPPH radical scavenging assay of the film**

It has been reported that the films were divided into small pieces and that 25 mg of the samples were dissolved in 3 mL of ethanol [18]. The film's antioxidant activity was then examined in the resultant combination. With a few minor adjustments to the sample preparation combination, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment was carried out in accordance with earlier procedures [22]. Equation utilised to compute the DPPH value after the absorbance was measured at 517 nm using a microplate reader (PowerWave x 340, Bio-Tek Instruments, USA) as showed by [23].

### **2.3.5.2. ABTS + scavenging activity of the film**

With certain slight modifications, the ABTS + scavenging activity of the film was evaluated using the methodology described by Seiquer et al. (2015). To be more precise, 990  $\mu\text{L}$  of diluted ABTS + solution was added to a 96-well microplate after 10  $\mu\text{L}$  of the disintegrated film samples. The plate was incubated at room temperature for six minutes in the dark. Equation 5 was utilised to compute the percentage of ABTS + scavenging activity using the measured value of absorbance at 734 nm.

## **2.4 Chicken meat samples Packaged with GF**

With very few modifications, active packaging films were used for wrapping samples of chicken meat in accordance with Farhan and Hani (2020). We acquired both pre-packaged and freshly cut chicken meat from Koley market in Kolkata, India. The meat was aseptically diced into cubes weighing  $30 \pm 3$  g. The samples of chicken meat were separated into four groups: pre-packaged chicken meat from the market (MF), packaged with GPO incorporated starch-based film (GF), packaged with a starch-based control film (SF), and Without film (WF). For a maximum of seven days, all samples were kept at a refrigerated temperature ( $3^{\circ}\text{C} \pm 1$ ) and relative humidity ( $80\% \pm 2$ ), with daily quality assessments conducted on days 0 to 7.

### **2.4.1. Physiochemical Analysis of Chicken Breast**

#### **2.4.1.1. Moisture Content of Chicken Breast**

According to AOAC METHOD, 1990, the moisture content of all the samples of chicken breast has been determined in this present study.

#### **2.4.1.2. pH of Chicken Breast**

For each procedure and storage day, the pH of the chicken meat sample was measured (Wazir et al., 2019). After homogenising the chicken sample pieces for one minute with distilled water (1:10), the homogenate's pH was determined with a digital pH metre (Seven Compact, Mettler Toledo, USA).

#### **2.4.1.3. Coliform count**

Samples of 10 g of chicken flesh were homogenised in 90 mL of sterile 0.1% peptone water (Merck, Darmstadt, Germany) after being aseptically removed from the packaging films. After homogenization, the samples were serially diluted using a 0.1% peptone water solution at a ratio of 1:10. Spreading 100  $\mu$ L of the diluted material onto plate count agar (PCA) and incubating it for 24 hours at  $37 \pm 1^\circ\text{C}$  yielded the total plate count (TPC). In microbiological counts, colony forming units (CFU/g) were expressed as  $\log_{10}$  values. A repeat analysis of every storage condition was conducted for microbial counts.

### **2.5. Statistical Analysis**

The data were represented as mean  $\pm$  standard deviation (SD) and were analysed using the Minitab statistical programme (Minitab 21.0, Minitab Incorporation, USA). An analysis of variance was performed to determine the significance ( $P=0.05$ ) of the differences within means (ANOVA).

## **3. RESULT AND DISCUSSION**

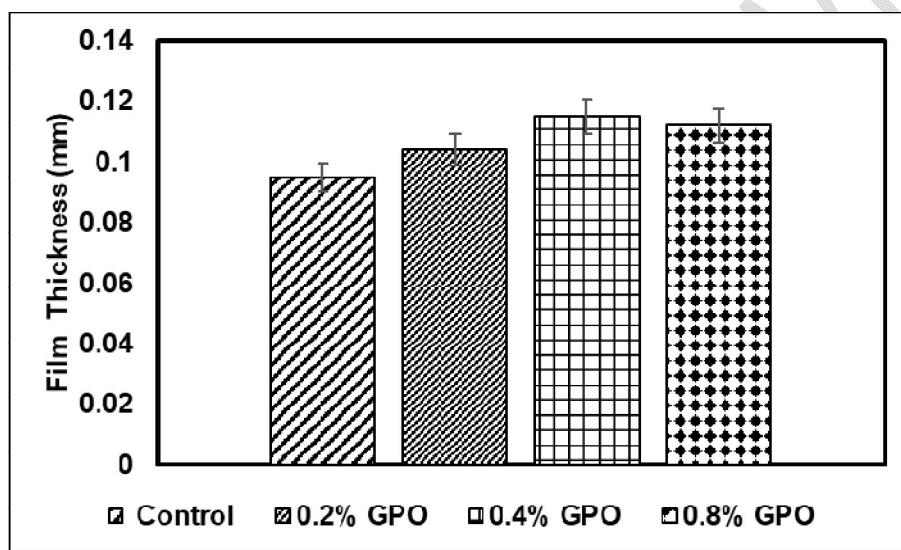
### **3.1. Physical Characteristics of the film**

#### **3.1.1. *Thickness of Film***

The properties of film-forming dispersions and films are considerably changed when essential oils (EOs) are added. A preliminary investigation showed that a starch-based film-forming solution becomes more fragile and difficult to handle when more than 0.8% of GPO is added. This is due to the fact that increased GPO concentrations have an impact on stability, size distribution, and particle size [24]. Although essential oils are generally accepted as safe (GRAS), larger amounts are not recommended because there is little research on the chemical interactions of GPO. In order to obtain significant bioactivity and physicochemical characteristics, a concentration range of 0.1% to 0.8% (w/v) is recommended.

Several essential oils have been included into biopolymer films in earlier research. For instance, fennel and cinnamon bark essential oils were added to cassava starch films at corresponding

quantities of 0.05% to 0.30% (v/v) [25]. Furthermore, fish gelatin films containing *Morindacitrifolia* oil were employed at concentrations ranging from 1% to 3% [18]. These experiments demonstrate how essential oils can improve the performance of biodegradable films; however, in order to prevent the integrity of the film from being compromised, attention must be taken in determining the precise interactions and optimum concentrations. Fig. 2 shows that the film thickness of the Gondhoraj peel based essential oil (GPO)-infused starch-based film and the control film does not differ significantly ( $p=0.05$ ). Furthermore, compared to the control film thickness of  $0.099 \pm 0.01$  mm, increasing the GPO concentration from 0.1% to 0.8% causes the film thickness to grow from  $0.103 \pm 0.01$  mm to  $0.112 \pm 0.01$  mm. Green tea and palm oil-infused cassava starch films showed a comparable increase in thickness with increasing oil concentrations [23].

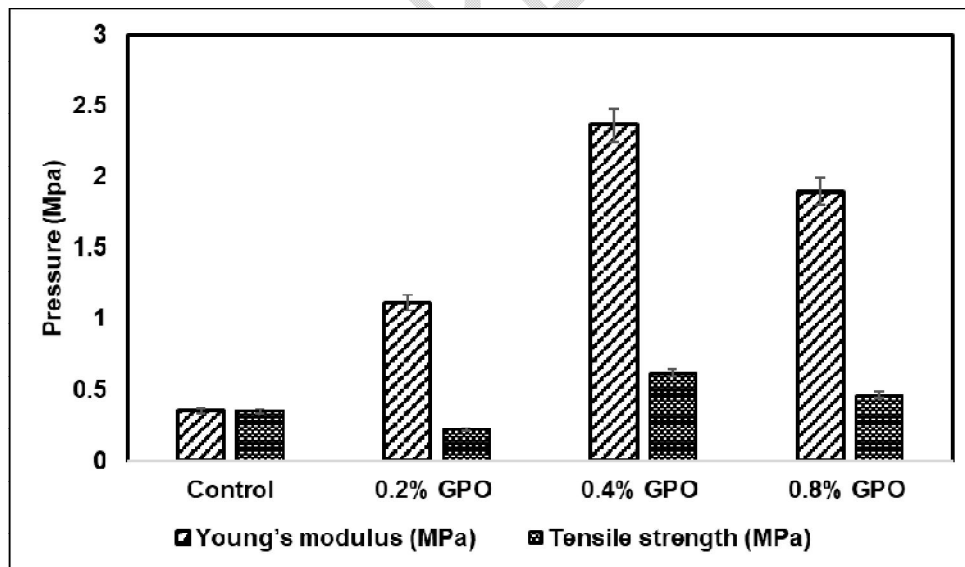


**Fig 2: Effect on film thickness of active packaging film after inclusion of GPO in starch film at different concentration in starch film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

### 3.1.2. Mechanical Properties of the Film

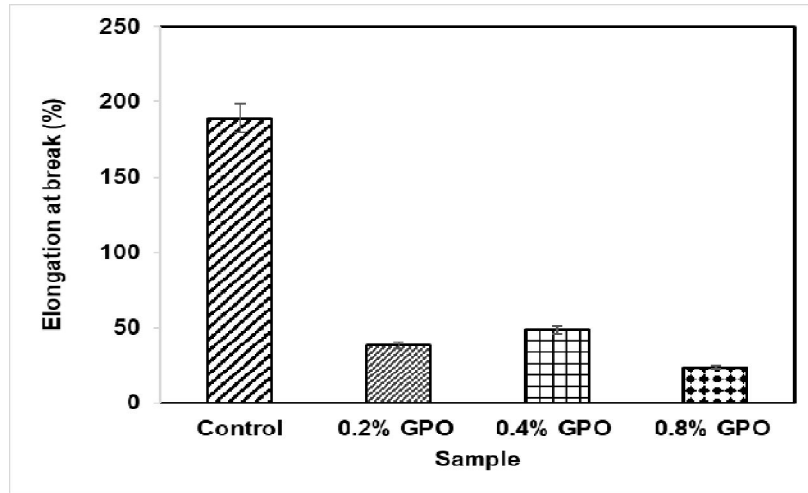
Tensile strength (TS), which may be determined with a universal testing machine, is an essential property that characterizes the mechanical properties of composite materials, like films [26]. Adding essential oil will significantly improve the mechanical characteristics and processing ability of starch sheets. Fig 3 shows that the films' TS varied between  $0.14 \pm 0.02$  MPa and  $0.63 \pm 0.02$  MPa. The film containing 0.2% gondhoraj peel essential oil (GPO) exhibited a reduced tensile strength (TS) in comparison to the control film. In a prior investigation, it has been discovered that the TS value of wheat starch film was lowered by the addition of lemon essential oil [27]. The films' Young's modulus (YM) increased by up to 0.4% with the addition of GPO, ranging from  $0.351 \pm 0.03$  MPa to  $2.915 \pm 0.05$  MPa. Nevertheless, a detrimental effect on YM was noted at 0.8% GPO. The rise in the volumetric share of grain borders and triple junctions, as well as the elastic deformation of grain boundaries, are responsible for the decrease in YM [28]. Since more flexible materials have lower elasticity modules and stiffer materials have higher ones, TGIEO starch-based films displayed greater YM values than the control Young's modulus, which measures the force of interatomic bonding, is largely determined by the chemical composition of the material and just somewhat by its microstructure morphology. It represents a material's relative stiffness or rigidity, which shouldn't be so low that it can't tolerate tearing.



**Fig 3: Effect of GPO Incorporation on Mechanical Characteristics of Active Packaging Film**

*Mean  $\pm$  S.E.M = Mean  $\pm$  Standard Error of means of three experiments*

The elongation at break (EAB) of the films increased from  $33.60 \pm 1.03\%$  to  $188.86 \pm 0.83\%$  with the addition of up to 0.4% GPO that is shown in Fig 4. This increase in EAB indicates a change in the starch structure, resulting in a less dense matrix. Consequently, the movement of polymer chains is facilitated under stress, demonstrating the film's enhanced elasticity [29].



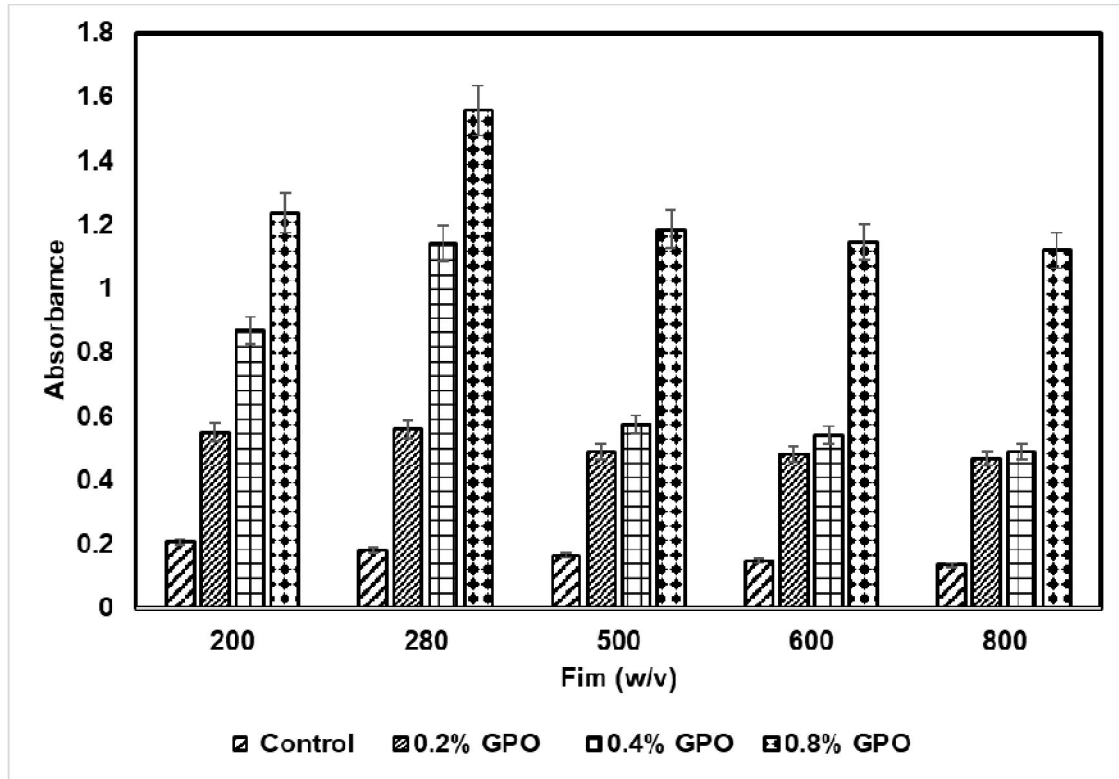
**Fig 4: Effect of GPO on Elongation Properties of Active Packaging Film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

### 3.1.3. Transmission of Light and Opacity

Fig. 5 shows that as the wavelength increased from 200 to 800 nm, the light transmission of the control film and the starch film containing GPO both increased. Unpleasant smells and odors can result from oxidation reactions in food products caused by light transmission, including UV and visible light. Thus, one of the most important roles of packaging material is to filter hazardous wavelengths [30]. When comparing the light transmission of the TGIEO starch-based film to the control film, a significant difference ( $p=0.05$ ) was found. As the quantity of GPO in the starch film was increased from 0.2% to 0.8%, light transmission decreased from  $33.92 \pm 0.00\%$  to  $0.00\%$  to  $7.58 \pm 0.03\%$ , respectively. The light transmission of the control film was 73.49% at 800 nm. The light scattering effect is probably what caused the film's transparency to diminish [31]. In comparison to the control film ( $1.620 \pm 0.00$ ), the film's opacity rose considerably ( $p=0.05$ ) with higher GPO concentrations (from  $3.150 \pm 0.03$  to  $8.924 \pm 0.09$ ) as shown in Figure 6. Increased opacity, which is caused by light scattering, shows that the sample is opaquer. The higher opacity and decreased light transmission are believed to be the result of this increased interaction between the phenolic chemical and starch [31].

Because of their limited water solubility, essential oils generate huge particle formations that scatter visible light, which increases opacity when added to edible coatings [32].

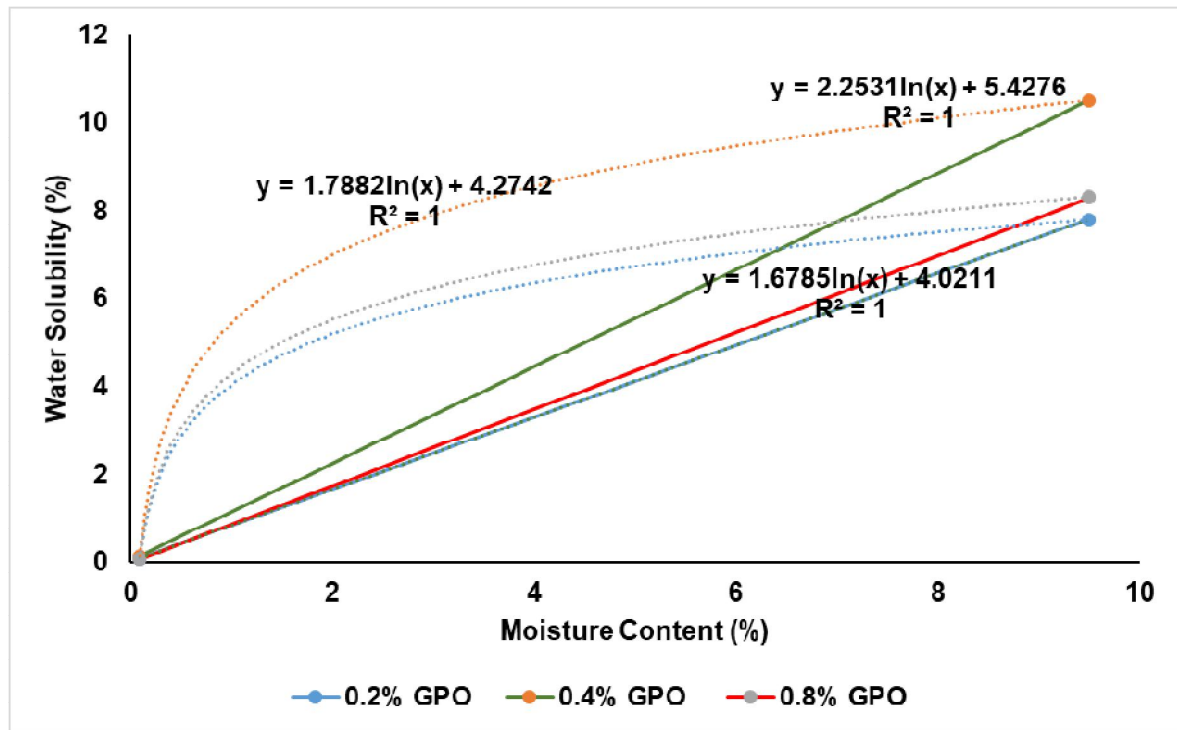


**Fig 5: Effect of GPO Concentration on Light Transmission Properties of Active Packaging Film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

### 3.1.4. Determination of moisture content and water solubility of the film

The GPO starch-based film's moisture content and water solubility were examined (Fig 6). The film's moisture content varied between  $7.76 \pm 0.08\%$  and  $10.48 \pm 0.35\%$ , with no discernible variation ( $p < 0.05$ ) when compared to the control film. 0.2% GPO had the lowest moisture content, which is advantageous for the film's possible application in packaging (Arezoo et al., 2020). When GPO was added to the starch-based film, its water solubility was considerably reduced (from  $0.107 \pm 0.13\%$  to  $0.068 \pm 0.09\%$  to  $0.090 \pm 0.00\%$ ). Since the hydrophobic essential oil limits the accessibility of hydroxyl groups in the starch, restricting polymer-water interactions, this reduction is most likely the result of interactions between the starch matrix and the essential oil [31].

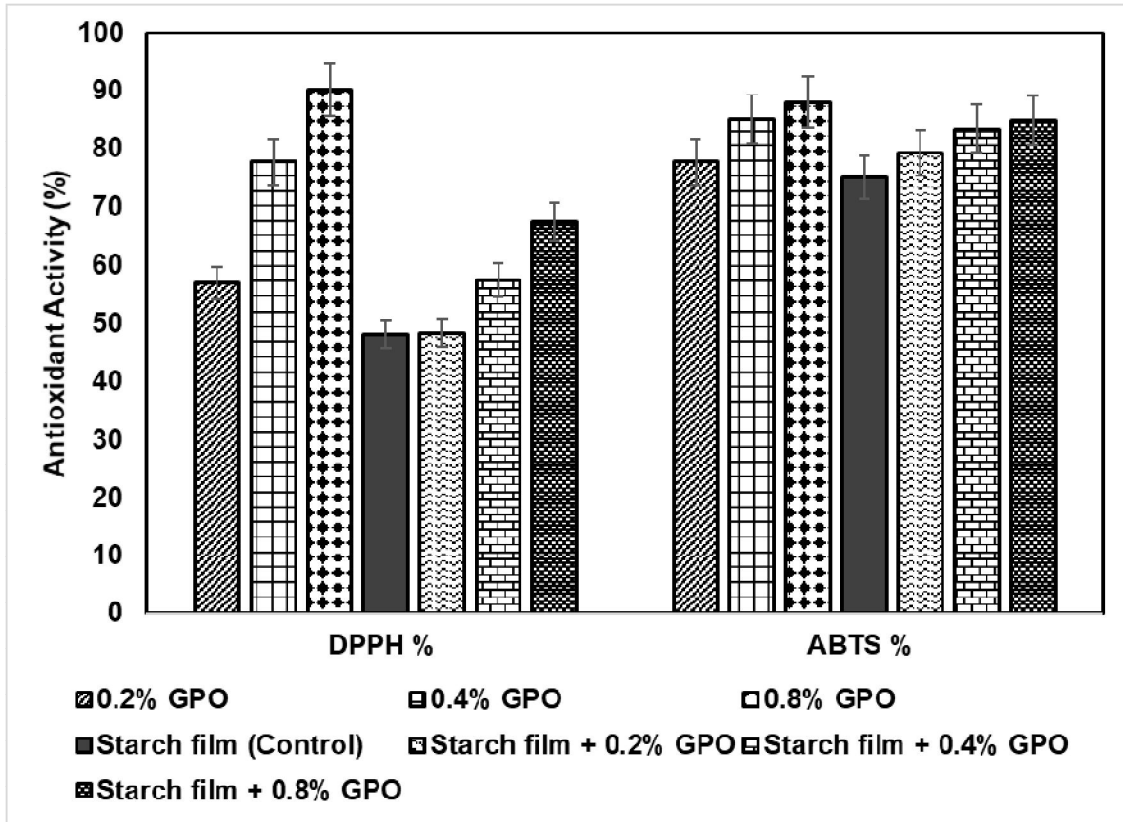


**Fig 6: Effect of GPO Concentration on Moisture content as well as water solubility of Active Packaging Film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

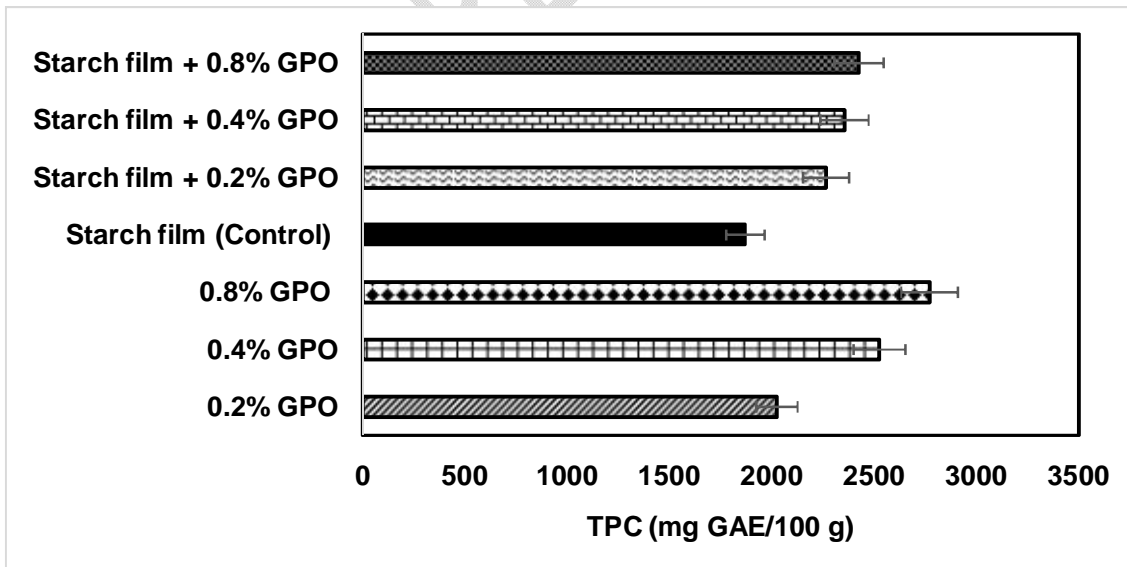
### 3.1.5. Antioxidant Properties of the film

Fig. 7 shows that the GPO starch-based film exhibited radical scavenging action, with  $70.36 \pm 2.48\%$  for DPPH and  $89.78 \pm 0.57\%$  for ABTS+ at the maximum GPO concentration of 0.8%. With increasing GPO concentrations, the films' antioxidant capabilities, including total phenolic content (TPC), increased significantly ( $p=0.05$ ) as shown in Fig. 8. A high recovery percentage of these chemicals suggests that the phenolic compounds in the polar fraction are responsible for this activity [24]. Because of the drying process and exposure to light, the antioxidant activity of the dried GPO starch-based films in this investigation was lower than that of the essential oil alone. Similar findings were noted when using *Morindacitrifolia* oil in active packaging [18], where interactions between the extract and film components influenced antioxidant activity. Furthermore, some essential oils are unable to participate in antioxidant activity responses because of their binding with the film matrix [16].



**Fig 7: Effect of GPO Concentration on Radical Scavenging Action of Active Packaging Film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*



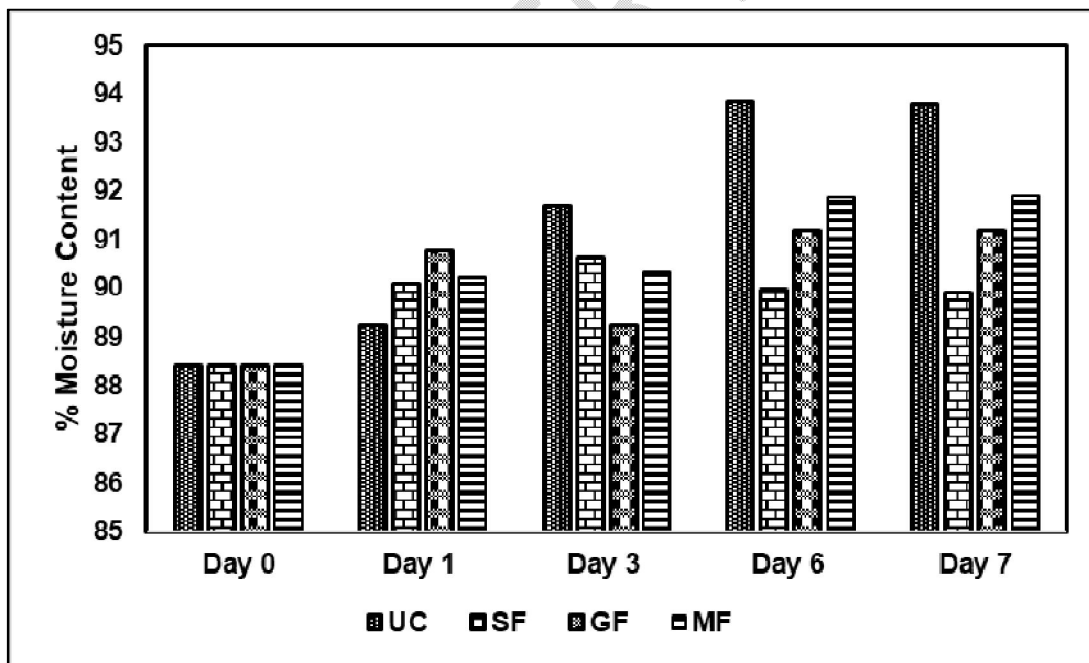
**Fig 8: Total Phenolic Content GPO and GPO infused Active Packaging Film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

### 3.2. Physicochemical Analysis of Chicken Breast

#### 3.2.1. *Moisture Content of Chicken Breast Packaged with active packaging film*

The physicochemical characteristics of GPO incorporated into a starch-based edible film were assessed in the study along with its effect on the quality of chicken flesh during chilled storage spanning Days (0, 1,3,5,7) as shown in Fig 9. A variety of preparation circumstances were applied to the chicken samples: uncoated (UC), packed with a control starch-based film (SF), packed with GPO-incorporated starch-based film (GF), and packed on the same day from the local market (MF). It is crucial to track the weight loss of chicken meat during storage in order to evaluate the profitability and quality of the product. For days 0, 1, 3, 5 and 7, there was not a significant difference ( $p=0.05$ ) in the percentage of moisture content in any of the chicken samples stored. On the other hand, by Day 7, the moisture content percentages of the following chicken samples differed significantly ( $p=0.05$ ): UC ( $93.78\% \pm 0.65$ ), SF ( $89.90\% \pm 0.54$ ), GF ( $91.18 \pm 0.17$ ), and MF ( $91.92\% \pm 0.54$ ).



**Fig 9:Moisture Content of Chicken Breast Packaged with active packaging film**

*Mean ± S.E.M = Mean ± Standard Error of means of three experiments*

### 3.2.2. pH of Chicken Breast Packaged with active packaging film

Corresponding with prior investigations, the chicken meat sample enclosed in the GPO starch-based film successfully restrained the rise in pH. According to that study, by preventing bacterial growth and protein denaturation, an active film comprising zinc oxide nanoparticles and chitosan nanofibers within a gelatin bio-nanocomposite film also reduced pH increase [30]. On the other hand, Day 7 pH values for the UC, SF, and MF chicken samples were significantly higher than Day 0 pH values ( $5.86 \pm 0.02$ ). At Day 7 of refrigerated storage, the pH of the chicken sample packed with the GPO starch-based film (TF) decreased significantly ( $p < 0.05$ ) in comparison to the chicken samples packed with UC ( $6.15 \pm 0.07$ ), SF ( $6.05 \pm 0.02$ ), and MF ( $6.24 \pm 0.07$ ) that showed in Fig 10. In line with a prior study, an active film containing zinc oxide nanoparticles incorporated into a gelatin bio-nanocomposite film with chitosan nanofiber inhibited pH increase in chicken meat during storage by preventing bacterial growth and protein denaturation. Meat deterioration raises pH because of alkaline reactions that produce amines and  $\text{NH}_3$  during the generation of free amino acids, which is fueled by microbial development [33]. A correlation has been observed between the rise in pH of chicken meat and the growth of bacteria. This can be explained by the denaturation of proteins and the build-up of amines and ammonia by psychotropic bacteria, which are frequently present in chicken meat during storage [12].

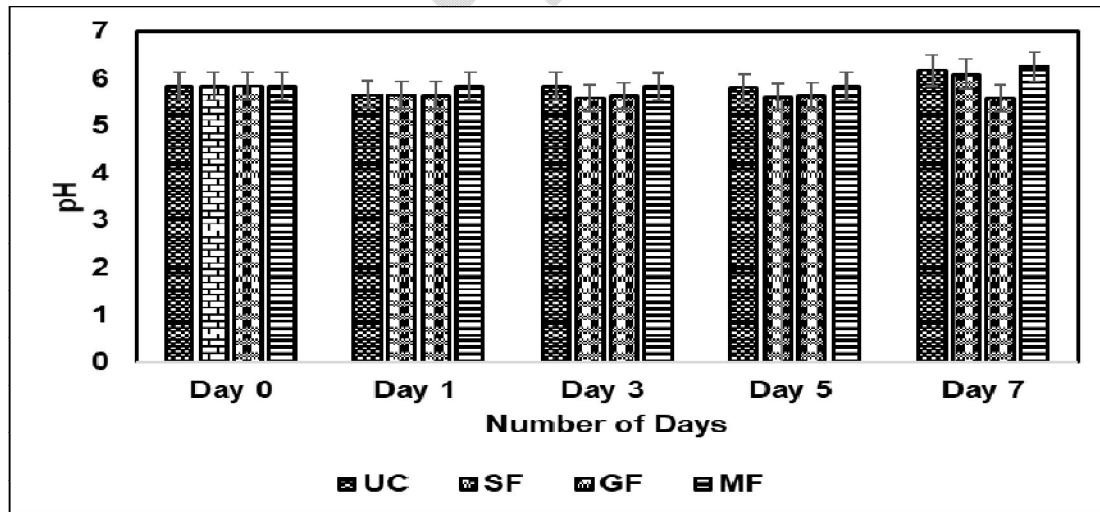


Fig 10: Change of pH of Chicken Chicken Breast Packaged with active packaging film

*Mean  $\pm$  S.E.M = Mean  $\pm$  Standard Error of means of three experiments*

### 3.2.3. Coliform Count of Chicken Breast Packaged with active packaging film

According to Fig 11, the first total bacterial count for fresh chicken breasts was  $1.72 \pm 0.02$  log CFU/g. According to ICMFS (1990), the recommended microbiological acceptability levels for raw chicken are 5-7 log CFU/g. However, Regulation EC 2073/05 (2005), which was passed in Spain, sets the limit at 106 CFU/g of meat. Compared to UC ( $6.73 \pm 0.05$  log CFU/g), SF ( $6.85 \pm 0.035$  log CFU/g), and MF ( $6.06 \pm 0.01$  log CFU/g), the chicken sample (TF) packed with GPO starch-based film significantly inhibited microbial growth, displaying the lowest microbial count ( $4.98 \pm 0.07$  log CFU/g) at the end of the chilled storage period. According to Muriel-Galet et al. (2015), antimicrobial agents decrease the quantity of live food-borne pathogens, lengthen the lag phase duration, and slow down the rate of microbial growth. To lengthen the shelf life of food goods, these antimicrobial chemicals are combined with polymers and released gradually [34].

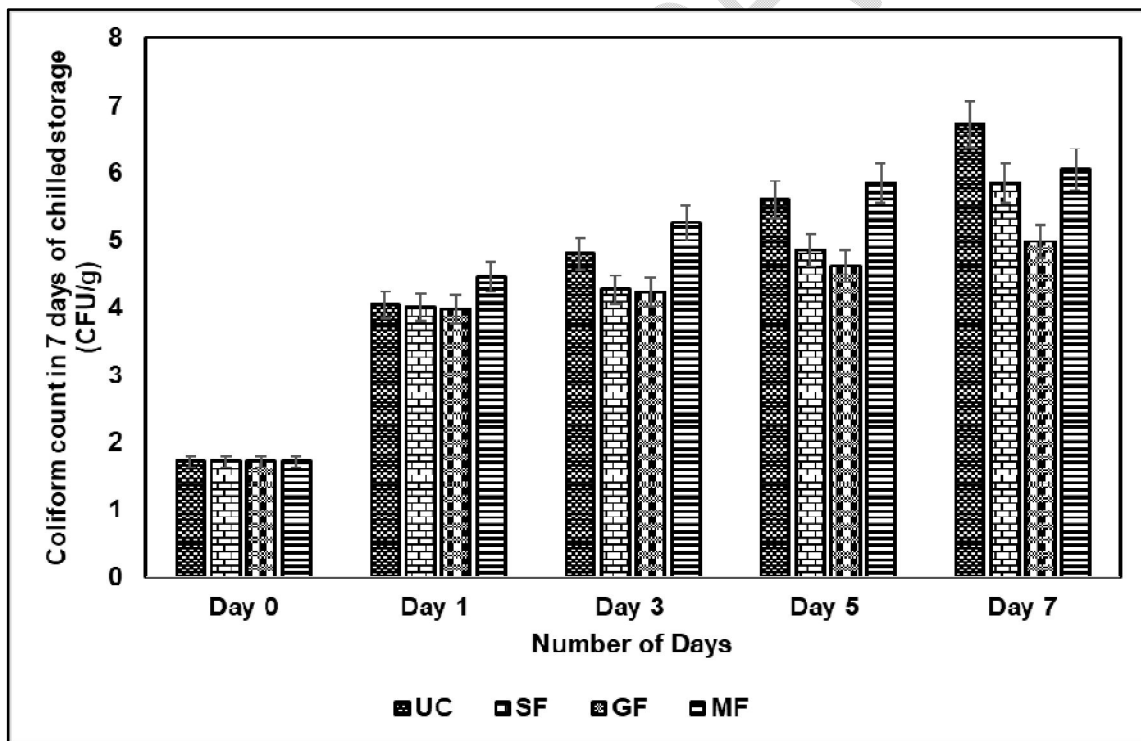


Fig 11: Coliform Count of Chicken Breast Packaged with active packaging film during storage period

*Mean  $\pm$  S.E.M = Mean  $\pm$  Standard Error of means of three experiments*

#### **4. CONCLUSION**

An approach to reducing or eliminating the usage of non-biodegradable packaging is to incorporate GPO into a starch-based film. This will lessen the impact on the environment and protect against chemical contamination. This study investigated the possibility of using GPO-infused starch-based film as a natural way to improve poultry meat safety and prolong its shelf life. During chilled storage, the chicken meat's quality and shelf life were significantly enhanced by the active packaging; at the conclusion of the storage period, the GF sample had the lowest TBARS and coliform counts. Significant antibacterial and antioxidant qualities were shown by the optimized hydro steam distillation extracted GPO in the starch-based edible film, which may help preserve the quality of chicken flesh in cold storage. Although this study focused on chicken meat, GPO starch-based active packaging could also be applied to other protein-rich foods such as fish and seafood.

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