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Development and Quality Evaluation of Edible Sachet Prepared with Potato Starch

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Plastics are made from petroleum and are difficult to biodegrade. Food packaging's goal is to keep food fresh throughout transportation from the factory to the client. Edible starch-based starches can be used to reduce the environmental impact of plastic packaging. The antioxidant impact of active films was evaluated using the peroxide index. In terms of physicochemical properties, the results showed that potato starch films beat synthetic polymers. These studies show that pectin may be combined with starch films to produce biodegradable films that can subsequently be used as active packaging for spices and other products. A large amount of starch or glycerin, on the other hand, may increase brittleness, so keep it modest.

Films based on potato starch were produced through a casting method. The film production process involved the addition of a plasticizer, resulting in the creation of several samples labeled as T1, T2, T3, and T4. A total of four samples with different compositions were generated and deemed suitable for subsequent analysis.. The results showed that the film labeled T3 had the highest thickness (0.300 mm) and transparency value (3.02), making it the most favorable among all the processed

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films. The pH of the films ranged from 5.85 to 6.73, and as the pectin concentration decreased, both water and moisture absorption decreased. However, additional research is needed before this material may be used as active food packaging.

Keywords: Edible film; pectin; glycerol; potato starch; spices; spice mix.

1. INTRODUCTION

Millions of tons of plastic are manufactured worldwide each year. Production and consumption increase year after year. Due to its vast range of applications, notably as a packaging material, it has become a widely used substance and plays an important role in our daily lives.

Petrochemical-based plastics such as polyolefins, polyesters, and polyamides are widely used in packaging materials due to their low cost, high volume availability, and favorable functional properties such as excellent tensile and tear strength, as well as good barrier properties against oxygen and aromatic compounds. These plastics, on the other hand, are made of petroleum-based components and are difficult to biodegrade, resulting in pollution and a serious ecological risk.

Biopolymer films are biodegradable and renewable, which reduces their environmental impact. While biomaterials have superior mechanical properties, they cannot completely replace synthetic packaging materials. Food packaging serves the function of keeping food fresh during transportation from the site of manufacture to the market and the buyer. To do so, the content must be protected from outside environmental factors. Food can be physically harmed when being stored, transported, or dropped or crushed at home [1]. Environmental variables such as water, gases, light, odors, or microorganisms can harm food if proper packing is not used [1].

Potato starch contains more phosphorus than other starches Jane [2].Starch granules do not dissolve in cold water. Excess water expands the starch granules, breaks the ordered structure at gelatinization temperature, and increases viscosity [3]. Agitation or mixing is required during the gelatinization process to increase the leaching of amylose and amylopectin into the water. This also inhibits the diffusion of amylose and amylopectin from the granules into the aqueous phase, resulting in partially gelatinized granules [4].

Pectin is used in the food industry as a stabilizer, thickener, gelling agent, anti-crystallizer, and encapsulating agent. They are used to create gels at low pH in the presence of divalent calcium. Food sector applications include low sugar jams and jellies, dairy desserts, fruit gel ice creams, fruit and vegetable syrup thickeners, and food coatings.

Another method for reducing moisture absorption in films is to use hydrophobic plasticizers. These plasticizers must be compatible with the polymers utilized in the film formation [5]. Glycerol and other low molecular weight polyhydroxy chemicals, polyethers, and urea are common plasticizers for starch-based films. Glycerol has been discovered to be the best plasticizer for keeping mechanical characteristics and transparency. However, the use of plasticizers affects not just mechanical qualities but also the barrier properties of the films. Unfortunately, adding plasticizers raises the hydrophilicity of the films, resulting in greater water vapor permeability. Biodegradable (PE) plastics bags own almost the same qualities as ordinary plastics bags. Biodegradable plastics bags differ mainly through its compostability (biological reduction) [6].

Glycerol is a popular plasticizer because of its stability and compatibility with hydrophilic starch chains. Glycerol's hydroxyl groups serve an important role in forming contacts (hydrogen bonds) both within and between polymer chains, resulting in a more flexible film structure. Vanin et al,[7]. studied the effect of plasticizers and their concentrations on the thermal and functional properties of gelatin based films. Four polyols (glycerol—GLY, propylene glycol—PPG, diDTG and ethylene glycol—ETG) were used in five concentrations: 10, 15, 20, 25, and 30 g plasticizer/100 g of gelatin to prepare the film. Plasticizer effect and efficiency were observed with DTG and ETG on the thermal properties, and with the GLY in terms of functional properties.

The primary goal of this study was to create active food packaging materials with natural edible components such as starch and pectin.

These materials strive to ensure the safety and healthiness of packaged foods while also increasing their shelf life. The study also emphasized the usage of biodegradable materials, which are environmentally benign, cost-effective, easily available, and capable of minimizing trash output. The studied Starch-based biodegradable mulching films showed good product performance, good crop quality and high yield in protected strawberry cultivation *Kapanen (2012)*

The high solubility of potato starch, pectin, and glycerol makes them ideal for use in filmmaking. Making edible sachets from these elements has the potential to reduce environmental pollution because they can be prepared alongside food, such as soup or vegetables. For example, an edible sachet made of potato starch can retain spices and be immediately utilized in foods such as soup, noodles, or pasta, giving consumers convenience while also improving the environment.

2. MATERIALS AND METHODS

2.1 Materials

Potato starch, pectin, glycerol, distilled water were obtained from FPE laboratory, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj, U.P, India. Spice mix was purchased from the local market of Prayagraj, U.P.

2.2 Methods

2.2.1 Preparation of film

Potato starch-based films were formed using a casting method. However, films produced without the addition of plasticizers were found to be too brittle for use in food packaging. To improve flexibility, several samples were created by incorporating a plasticizer into the film production process and were labeled as T1, T2, T3, and T4.

Active films are commonly employed in packaging systems for food, pharmaceuticals, and various other products. Their functionalities include extending shelf life, monitoring freshness, displaying quality information, enhancing safety, and providing convenience.

A total of four samples with varying compositions were prepared and found to be suitable for further investigation. These plastic film samples underwent property testing in accordance with the Plastic Food Packaging Code to assess their characteristics and suitability for potential applications in food packaging and related industries. Further analysis and research may be conducted based on the results obtained from these property tests to optimize the potato starch-based films with plasticizers.

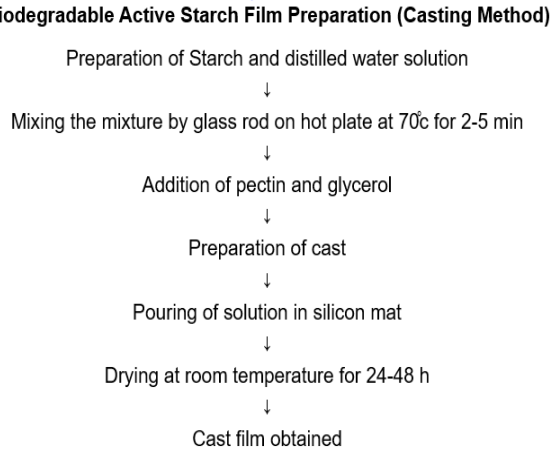

Fig. 1. Biodegradable active starch film preparation (casting methods)

2.2.2 Thickness of potato starch film

The thickness of each film sample was measured using a micrometer. Four different points on each film were tested to determine the average caliper, and this average value was calculated. Film thickness is a crucial parameter as it can influence the strength and various other properties of packaging films.

2.2.3 Transparency of potato starch film

The test to assess transparency was conducted following the standard test method for transparency of plastic sheeting (ASTM method D1746). Rectangular pieces of the film samples were cut and directly placed in a spectrophotometer test cell to capture the absorbance spectrum. Air was used as the reference value for the measurements.

The transparency value of each film was then calculated using

$$T = A600/M \qquad \text{Eqn 1}$$

Where, T is the transparency value A600 is the absorbance at 600 nm M is the thickness of the

films in millimeters. According to this equation, a higher value of T indicates a lower degree of transparency, meaning that films with a higher T value are less transparent.

2.2.4 pH of potato starch film

The pH of the film samples was measured using the method outlined in Ranganna's work (2000). Distilled water was placed in a beaker, and the pH meter's electrode was immersed in the water. The pH meter was manually adjusted using a knob until the reading displayed 7, representing the neutral pH value.

Afterward, the film samples were placed in another beaker, and their pH values were measured by immersing the electrodes of a digital pH meter into the beaker.

2.2.5 Test for water absorption of potato starch film

The films were cut into pieces measuring 2.5 x 2.5 cm and weighed under air-dry conditions (W1). Next, they were soaked in distilled water at a temperature of 25 °C for 30 minutes. After soaking, the wet samples were gently wiped with filter paper to remove excess liquid and then reweighed (W2). The films were then kept in distilled water for additional durations of 30, 60, 120, and 240 minutes.

To determine the amount of water absorbed by the films, the following formula was used:

$$\% = 100 * (W2 - W1)/W1 \qquad \text{Eq 2}$$

In this equation, W2 represents the weight of the wet sample, and W1 represents the weight of the air-dried sample (Bigi et al., 2004). The measurements were repeated three times for each type of film, and the average value was taken as the final result.

2.2.6 Test for biodegradability of potato starch film

To assess the biodegradability of each film sample, a modified version of the test described in the article by Azahari et al. [8] was conducted. Several small pots were filled with composted soil, and each film sample was cut into pieces measuring 2 cm x 2 cm. These film pieces were buried in the soil to a depth of 5 cm. The pots were placed in the laboratory and regularly watered to maintain moist soil conditions. Any excess water was drained through holes in the bottom of the pots.

Over several days, the samples were left in the soil to study the biodegradation process. At specific intervals (e.g., every five days), the samples were gently removed from the pots, cleaned to remove any dirt, and then dried until a constant weight was achieved. The weight loss of the samples over time was measured to analyze the degradation kinetics during the soil burying tests.

2.2.7 Test for moisture permeability of potato starch films

For this test, 1 gram of salt was encapsulated within a piece of biodegradable plastic film. The plastic films were all cut to the same dimensions, measuring 4 cm x 4 cm.

The encapsulated salt samples were then exposed to natural air for a duration of 30 days. After this period, the amount of moisture absorbed by the salt was measured using Equation 3:

$$\% = (Final\ weight\ of\ the\ salt - Initial\ weight\ of\ salt) \times 100 \div Initial\ weight\ of\ salt \qquad \text{Eqn 3}$$

This formula calculates the percentage of moisture gained by the salt during the 30-day exposure period.

2.3 Scanning Electron Microscopy (SEM) Image of Potato Starch Film

The films were prepared for observation using scanning electron microscopy (SEM) as follows: small pieces of the films, measuring 10 x 10 mm, were cut and dried. These dried film pieces were then mounted on aluminum stubs using double-sided carbon tape. To enhance their surface conductivity and improve imaging, a thin layer of gold was sputtered onto the film samples.

Observations of the surface and cross-sectional microstructures of the dried films were carried out using a scanning electron microscope (JSM-6510LV-LGS, JEOL Co., Ltd. USA) at MNIT Allahabad. During the SEM examination, all samples were subjected to an acceleration voltage of 20 KV, and the magnification was set

to 1000x. The resulting images were captured and analyzed to evaluate the homogeneity of the films.

2.4 X-Ray Diffraction of Potato Starch Film

The X-ray patterns of the potato starch composite sheets were examined using X-ray diffraction with Cu K-alpha radiation (Rigaku D/Max-IIIA, Tokyo, Japan) at MNNIT, Allahabad. The X-ray diffraction analysis was conducted at a voltage of 30 kV and 20 mA in a rice field environment. The sample was scanned over a range of 2 theta (2θ) values from 3 to 60 degrees at a scanning speed of 2 degrees per minute. Before testing, all film samples were stored in a desiccator to maintain their dry condition.

2.5 Peroxide Index (PI) of Potato Starch Film

The Peroxide Index (PI) was measured using the titration method specified by the Association of Official Analytical Chemists. Detailed instructions for the peroxide testing can be found in Appendix B. The analysis was conducted at the NABL laboratory located at the Food Technology Center-Professional Research Laboratory of Allahabad University.

2.6 Statistical Analysis of Potato Starch Film

The experiment was conducted using a fully randomized design. Data recorded during the course of the study were statistically analyzed by analysis of variance. R.A. Fisher in 1923. There is a convenient method that can be used to analyze the variability of population variances. A significant effect of treatment was assessed using F (variance ratio). Calculated F values were compared with table values for F at the 5% significance level. Impact was considered significant if the calculated value exceeded the tabulated value. Study significance was tested at the 5% level. The one-way ANOVA used for statistical analysis of the data is shown in Table 1.

where

TrSS: Sum of Squares for the "Treatment" or the variability between treatment groups.

MTrSS: Denotes the Mean Sum of Squares for "Treatment."

MESS: Represents the Mean Sum of Squares for "Error."

ESS:Stands for the Sum of Squares for "Error."

TSS: Represents the Total Sum of Squares

F-value: The Variance Ratio, also known as the F-value, is the ratio of the Mean Sum of Squares for "Treatment" to the Mean Sum of Squares for "Error."

df (Degree of Freedom):Represents the degrees of freedom associated with each source of variance. For "Treatment," it is denoted as (t-1), where t is the number of treatment groups, and for "Error," it is (t-1)r, where r is the number of replicates or observations within each treatment.

3. RESULTS AND DISCUSSION

3.1 Physico - Chemical Properties of Edible Film

3.1.1 Preparation of edible sachet using potato starch

Potato starch was obtained from the department laboratory and used to create biodegradable packaging. Glycerol was employed as the plasticizer for the starch. Different formulations were prepared with varying percentages of starch (4.42%, 6.54%, 8.81%, and 10.21%) and glycerol (3.96%, 3.49%, 1.32%, and 2.55%), along with the inclusion of pectin. The films were then formed using the casting method.

The progression of Potato starch film is depicted in various stages as illustrated below. Fig. 2 illustrates the initial process of creating the film solution, while Fig. 3 displays the starch film cast onto the silicon mat before peeling. The peeled final Potato starch film is presented in Fig. 4, and Fig. 5 showcases the end product where the spice mix is enclosed within the edible package crafted from Potato starch.

In the research, samples T2 and T3 were found to have an acceptable thickness, whereas sample T1 had an average thickness of 316 µm. The thickness of the film was influenced by the concentration of potato starch used in each sample. The thickness directly affects the film's water absorption behavior.

Table 1. One-way ANOVA

Source of Variance	Degree of Freedom	Sum of Square	Mean sum of Square	Variance ratio(F-value calculated)
Treatment	(t-1)	TrSS	TrSS/ (t-1) =MT	MTrSs MESS
Error	(t-1)r	ESS	ESS / (t-1)r=ME	-
Total	(rt-1)	TSS	-	-

Fig. 2 Potato starch film solution

Fig. 3. film casted in silicon mat

Fig. 4. Potato starch film after peeling

Fig. 5. Edible sachet containing spice mix potato starch film thickness

As mentioned earlier, T1 had the maximum thickness, while T2 and T3 exhibited thicknesses that were deemed suitable for sealing and overall film performance, as further analyzed and discussed.

However, it is important to note that the selection of the best sample couldn't be solely determined based on thickness alone. Other parameters also had to be taken into consideration for comprehensive evaluation. The readings and data for all samples are provided in Table 2, and they are graphically represented in Fig. 6.

3.2 Water Absorption of the Potato Starch Film

A water absorption test was conducted on samples T1, T2, and T3. Initially, the films were cut into small squares and weighed. These film samples were then immersed in various aqueous solutions, as specified in Table 3. After 30 minutes, 60 minutes, 120 minutes, and 240 minutes, the weights of the film samples were measured again. The percentage increase in weight for each sample was calculated and recorded as a measure of water absorption.

The water absorption rates of the films were represented in Fig. 7. It was observed that sample T3 exhibited the lowest water absorption compared to the other two samples, T1 and T2. The statistical analysis, as shown in ANOVA Table A.2, revealed that the calculated F value was greater than the critical value at a 5% probability level. This suggests a significant effect of treatment on the water absorption of the samples. In conclusion, treatment T3 displayed the least water absorption among all the treatments, indicating its superior performance in terms of water resistance compared to the other treatments (T1 and T2).

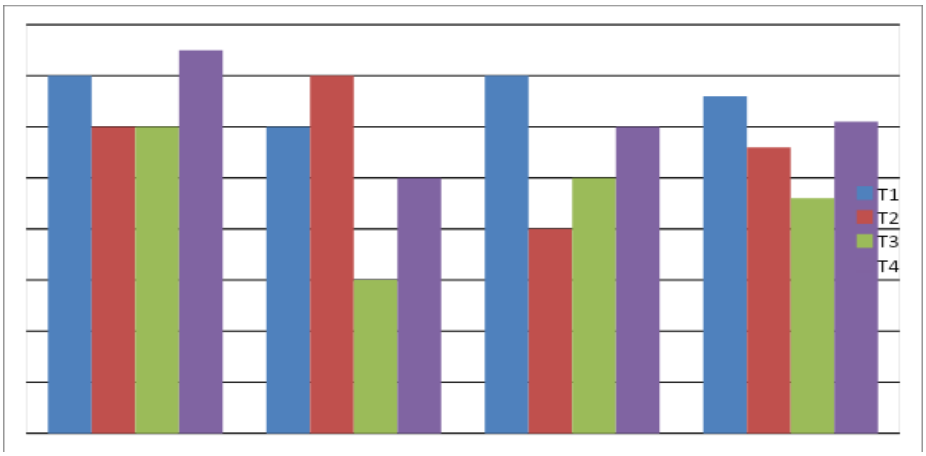

Fig. 6. Thickness of potato starch film (µm)

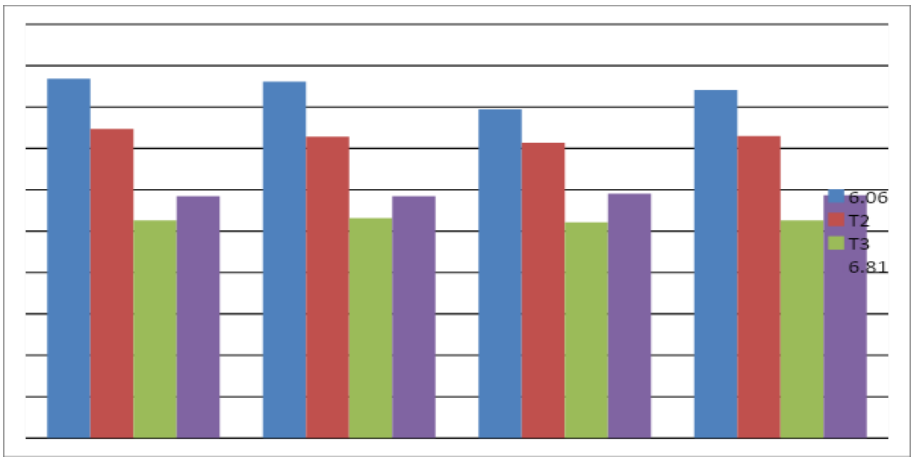

Fig. 7. Water absorption of the potato starch film (%) biodegradability of the potato starch film

Table 2. Thickness of the film

Treatments	R1	R2	R3	Mean
T1	0.320	0.310	0.320	0.316
T2	0.310	0.320	0.290	0.306
T3	0.310	0.280	0.300	0.296
T4	0.325	0.300	0.310	0.311
S.Ed (±)	Result of F test		Critical Difference at 5%	
(0.0070)	Treatments found Significant at 5% level of significance.		(0.015)	

Table 3. Water absorption of the potato starch film

Treatments	R1	R2	R3	Mean
T1	43.41	43.09	39.74	42.08
T2	37.36	36.41	35.67	36.48
T3	26.32	26.59	26.07	26.32
T4	29.24	29.21	29.54	29.33
S.Ed. (±)	Result of F test		Critical Difference at 5%	
(0.57)	Treatments found Significant at 5% level of significance.		(1.29)	

Two grams of each sample were analyzed to determine the percentage of weight loss. All the relevant biodegradation measurements are provided in Table 4.

The findings from this research are in alignment with similar results reported by Xiong et al 2008. in their studies on the structure and properties of starch-based biodegradable films, as well as biodegradation studies of polyvinyl alcohol/corn starch blend films in solid and solution media in 2008.

3.3 Moisture Permeability of Potato Starch Film

The moisture gains, determined from the salt loading of the specimens, are presented in the data, and the corresponding plots are depicted in Fig. 8. To evaluate the hygroscopic nature of salt, it was packed in foil for the testing process.

The surface pH values of the films ranged from 5.85 to 6.73. Table 6 provides the pH values for the four treated films (T1, T2, T3, and T4), and Fig. 9 graphically represents this data.

For approximate measurements, references were taken from the works of Ferrier et al. (2002), who conducted research on modified atmosphere packaging of minimally processed mango and pineapple fruits, and Choudhary et al. (2012), who investigated the possibility of developing fast-dissolving films from ondansetron hydrochloride.

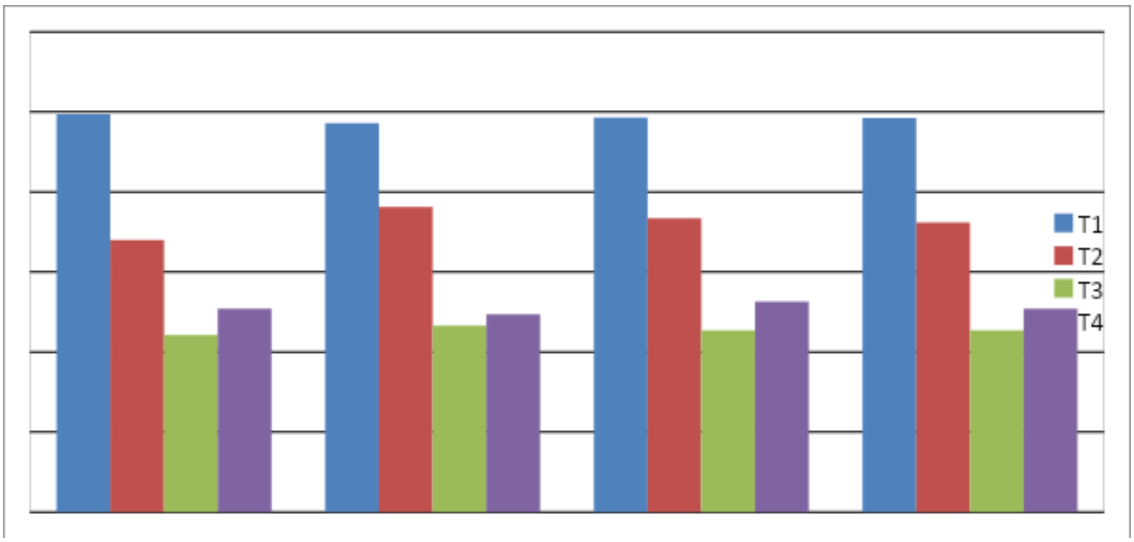

Fig 8. Moisture Permeability of Potato Starch Film Ph of Potato Starch Film

Table 4. Biodegradability of Potato Starch Films

Treatments (Days)	4th	6th	15th	30th
T1	85	100	-	-
T2	84	100	-	-
T3	89	100	-	-
T4	87	100	-	-

Table 5. Moisture permeability of the potato starch film (%)

Treatments	R1	R2	R3	Mean (%)
T1	4.97	4.86	4.93	4.92
T2	3.40	3.81	3.67	3.62
T3	2.21	2.33	2.27	2.27
T4	2.54	2.47	2.63	2.54
S.Ed. (±)	Result of F test		Critical Difference at 5%	
(0.071)	Treatments found Significant at 5% level of significance		(0.160)	

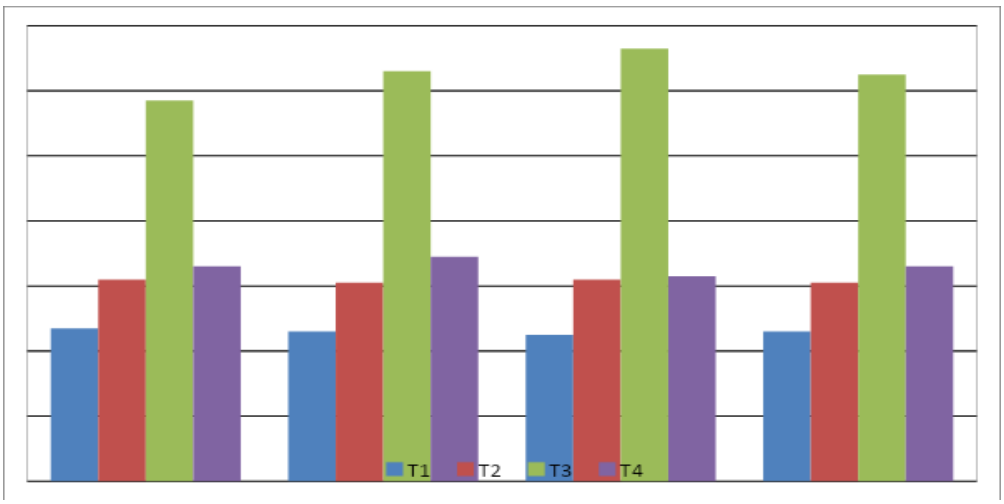

Fig. 9. Ph of Potato Starch Film

Table 6. pH of Potato Starch Films

Treatments	R1	R2	R3	Mean
T1	5.87	5.86	5.85	5.86
T2	6.02	6.01	6.02	6.01
T3	6.57	6.66	6.73	6.65
T4	6.06	6.09	6.03	6.06
S.Ed. (±)	Result of F test		Critical Difference at 5%	
(0.026)	Treatments found Significant at 5% level of significance.		(0.061)	

3.4 Transparency of the Potato Starch Film

The transparency of the film samples was assessed using a spectrophotometer, and it was observed that film sample T3 exhibited the highest level of transparency. In this context, a higher T value indicates a less transparent and more opaque sample. The film labeled T3 demonstrated the most clarity among all the treated films (T1, T2, T3, and T4). The transparency data for these films are presented in Table 10.

Similar findings were reported by Wang et al. (2016) in their study on the physical cross-linking of edible collagen casings, which also demonstrated the influence of different treatments on transparency in their respective samples.

Table 7. Transparency of potato starch films (%)

Treatments	R1	R2	R3	Mean (%)
T1	6.31	6.57	6.54	6.47
T2	5.09	5.12	5.15	5.12
T3	3.02	3.09	3.11	3.07
T4	4.65	4.65	4.70	4.66
S.Ed. (±)	Result of F test		Critical Difference at 5%	
(0.0348)	Treatments found Significant at 5% level of significance.		(0.078)	

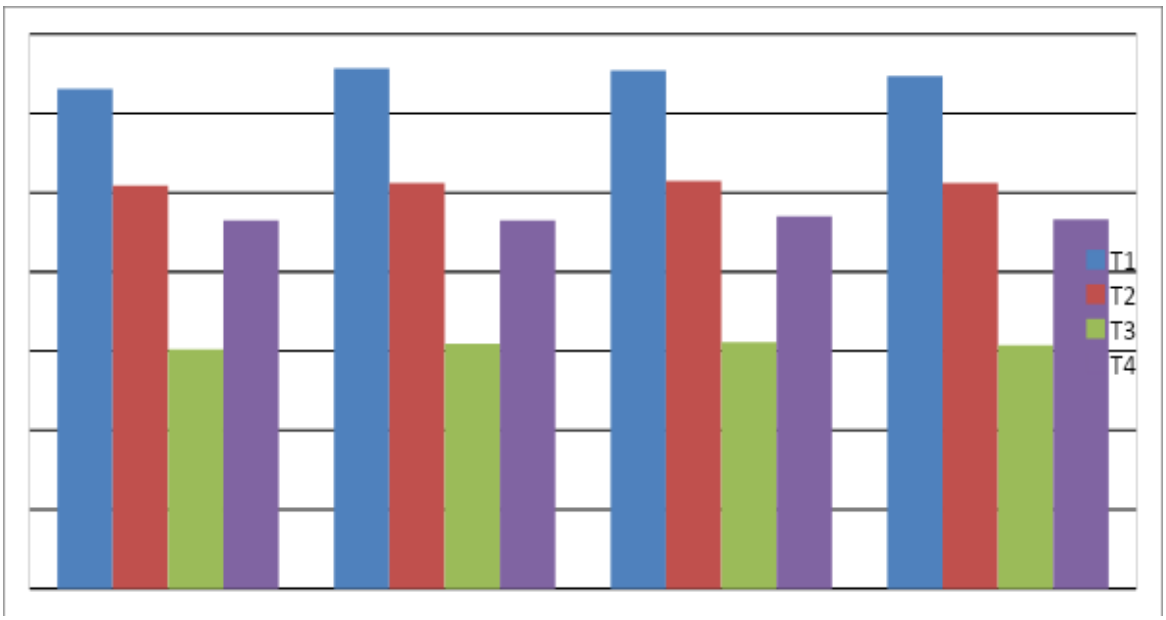

Fig 10. Transparency of the Potato Starch Film

3.5 Scanning Electron Microscopy (SEM) Image of Potato Starch Film

Scanning Electron Microscopy (SEM) was utilized to examine the surface morphology and cross-section of the films, assessing the homogeneity, presence of voids, and overall structure. Fig. 11, 12, 13, and 14 display the surface and cross-sectional morphologies of the films. Fig. 13 and 14 demonstrate that films F2 and F3 possess a flat, smooth appearance with a good compact structure, indicating the homogeneity of the mixture of starch, glycerin, and pectin in these films. However, the incorporation of pectin into starch resulted in heterogeneity in the starch matrix, as indicated by the appearance of white spots in Fig. 11 and 12. Fig. 10 and 11 also reveal the presence of numerous plaques and noticeable pores, disrupting the internal structure of film T1, which in turn affects its permeability.

Overall, these observations suggest that films with lower pectin and glycerin concentrations (T3) exhibit better mechanical and barrier properties compared to those with higher concentrations (T1 and T2). The results are consistent with the notion that surface properties play a crucial role in determining the barrier properties of films, favoring films with homogeneous and smooth surfaces (Wang et al., 2013). Additionally, the water permeability and moisture sensitivity of edible films are directly influenced by their surface properties and hydrophobicity.

3.6 X-Ray Diffraction of Potato Starch Film

Glycerol, used as a plasticizer in the film formulation, may have prevented starch molecules from entering the interlaminar spaces of the clay, possibly covering the entire interlaminar space. Nonetheless, non-volatile plasticizers are essential for processing practical starch-based materials. They are crucial in keeping the mixture of starch and pectin powder together after the water has evaporated, as explained by Chen and Evans (2005).

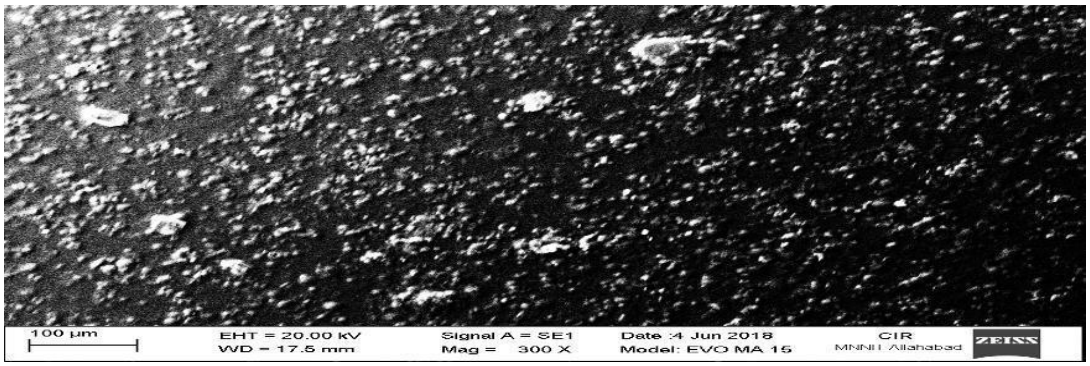

Fig. 11. SEM image of T1

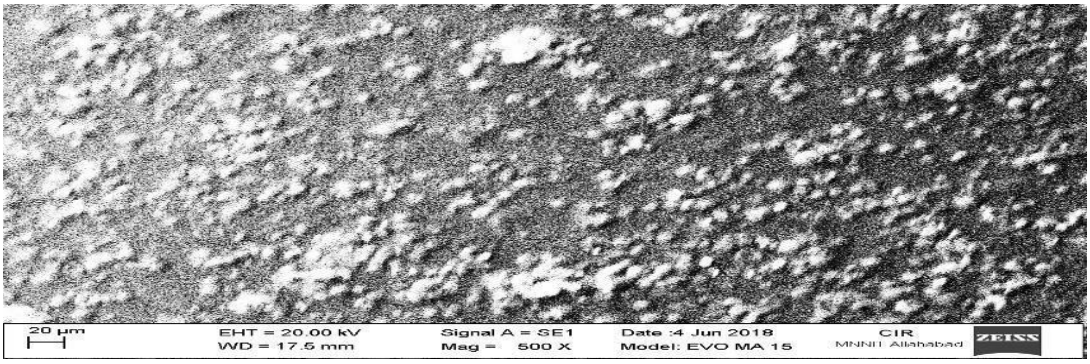

Fig. 12. SEM image of T2

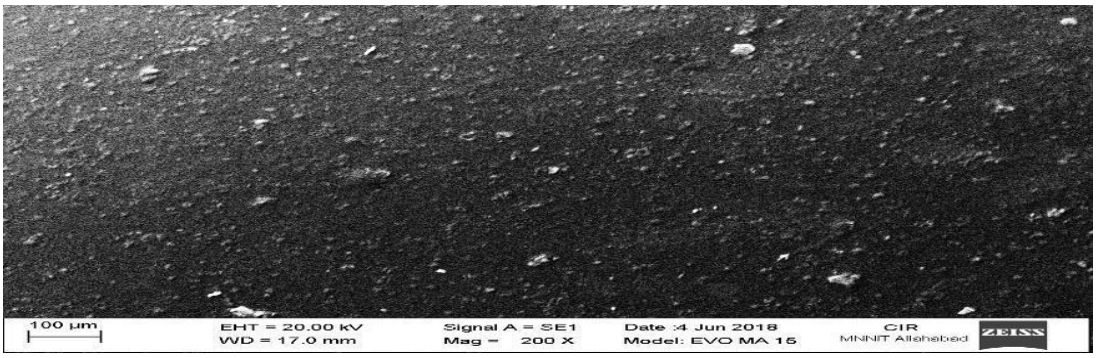

Fig. 13. SEM image of T3

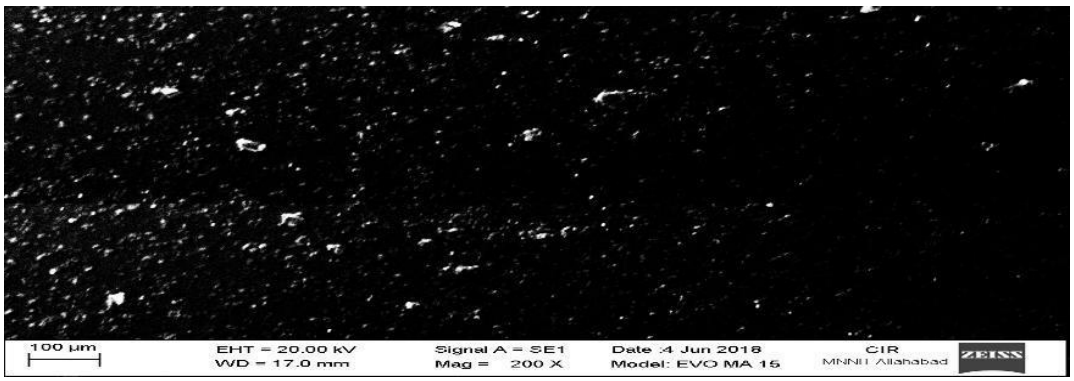

Fig. 14. SEM image of T4

3.7 Peroxide Index of Potato Starch Film

The peroxide value is a measure of the amount of oxygen peroxide per kilogram of fat in a substance. As the peroxide value increases, it indicates a higher level of rancidity. In the case of spices packaged with different packaging materials, the peroxide values showed a notable increase. Among them, the smallest increase was observed in spices wrapped with starch film containing 1.67% pectin. The detailed results are presented in Table 8, and Fig. 15 provides a graphical representation of the findings.

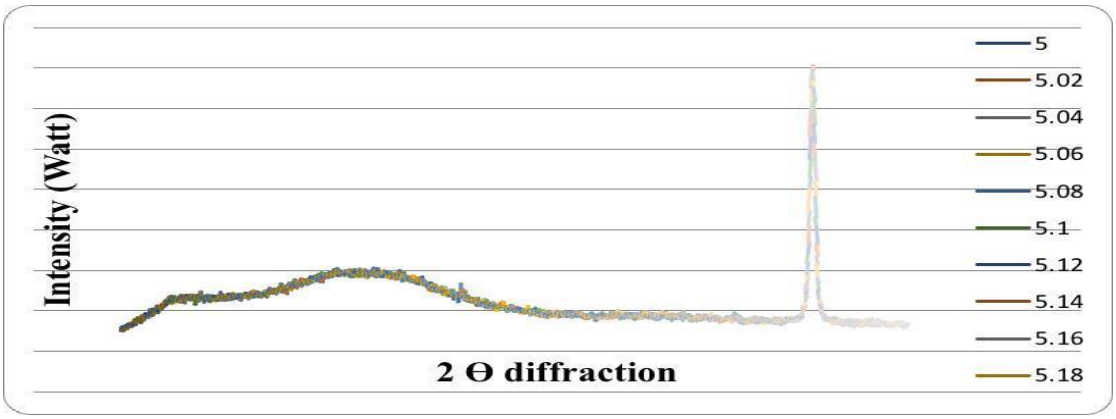

Fig. 15. X-Ray Diffraction of the Potato Starch Film

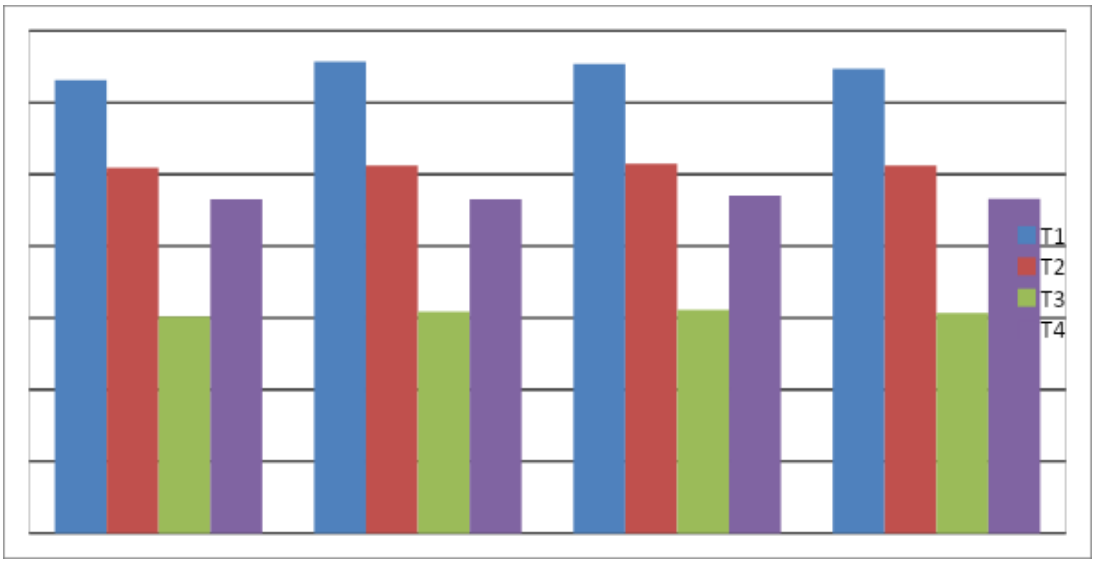

Fig. 16. Peroxide Index (%) of Potato Starch Film

Table 8. peroxide Index of potato starch films

Treatments	0-7th day	0-15th day	0 -21st day	Mean
T1	6.59	6.88	6.97	6.81
T2	5.43	5.59	5.68	5.56
T3	2.69	2.78	2.86	2.78
T4	4.41	4.55	4.97	4.64
S.Ed. (±)	Result of F test		Critical Difference at 5%	
0.059678	Treatments found Significant at 5% level of significance		0.135001	

4. SUMMARY AND CONCLUSION

The study involved the preparation of potato starch films incorporating pectin and glycerol using the casting method. The films were then evaluated for various characteristics, including thickness, transparency, pH, water absorption, moisture absorption, hygroscopicity, biodegradability, and structural properties using SEM and X-ray diffraction.

The results showed that the film labeled T3 had the highest thickness (0.300 mm) and transparency value (3.02), making it the most favorable among all the processed films. The pH of the films ranged from 5.85 to 6.73, and as the pectin concentration decreased, both water and moisture absorption decreased.

The active films were further assessed by packing them with spices and storing them for 21 days. The stability of the spices was measured by calculating the total peroxide value, and film T3 exhibited the minimal reduction, indicating better performance as an antioxidant.

Overall, the active films developed from potato starch with varying concentrations of pectin proved to be biodegradable and showed favorable physicochemical properties compared to synthetic plastics. These findings suggest that pectin can be incorporated into starch films to produce biodegradable materials suitable for active packaging of spices and other products. However, it is important to be cautious about using high concentrations of starch or glycerol as they may increase brittleness. Further research is necessary to validate the effectiveness of these films as active food packaging.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

B.1 To determine peroxide value of packed spices.

Methods

The peroxide value of an oil or fat was the amount of peroxides present and expressed as milli equivalents of peroxides per 1000 g of sample. The sample was dissolved in the solvent, treated with potassium iodide and the iodine liberated by the peroxide present in rancid fat or oil was titrated with sodium thiosulphate solution often the number of millimoles of peroxide oxygen was reported and the result was then half that of peroxide value. In this case the term ‗Lea Value Had been frequently used.

REAGENTS

1. Solvent: Two volumes of glacial acetic acid and one volume of chloroform were mixed. 2. Saturated Potassium Iodide Solution: Four parts of pure potassium iodide in three parts of distilled water were dissolved, and then the solution was kept in the bottle. 3. 0.1N Sodium thiosulphate solution.
4. 0.5% starch indicator.

PROCEDURE

An aliquot of extracted fat of the sample was weighed in a conical flask. 25 ml of solvent was added and the air above the liquid was displaced with CO2. 1 ml of potassium iodide solution was added. The flask was corked and was allowed to stand for 1 min (shaking) then 35 ml of water was added and a titration of the liberated iodine with 0.1 N sodium thiosulphate solution was done using starch indicator vigorous shaking at the end to remove the last traces of iodine from the chloroform layer was performed. The blank determination was carried out simultaneously. The thiosulphate consumption was negligible. The calculations were done according to Eq. A.

CALCULATION

Peroxide value (milliequivalents or millimoles per 1000 g of fat) = $(sample\ titre - blank\ titre) \times normality\ of\ thiosulfate\ solution\ /weight\ of\ fat\ taken(g) \times 1000$

Table A.6 Table for ANOVA of peroxide index of spices

Source	D.F	SS	MSS	Cal. F	TAB F(5%)	TAB F(1%)
Treatment	3	34.64577	11.54859	1621329	S	3.862548
Replication	3	0.231219	0.077073	10.82042	S	6.991917
Error	9	0.064106	0.007123	-	-	-
Total	15	34.94109	-	-	-	-
S.EM=	0.042199	CD(5%)= 0.135001	-	-	-	-
S. Ed	0.059678	CD (1%)= 0.193944	-	-	-	-

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