Development of Biodegradable and Anti-Microbial Film from Potato Starch and Garlic Extract using different Plasticizers

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ABSTRACT

Nowadays, biodegradable food packaging is becoming progressively important in the food industries, where advances in functionality such as convenience and portioning are gaining more attention. Furthermore, there is also an increased awareness on sustainability due to increasing environmental concerns. Researchers' interest has been increased more towards the formulation and development of bioplastics for the food packaging and distribution. This research study aims to develop biodegradable and antimicrobial film from potato starch and garlic extract using different plasticizers namely glycerol, honey, and beeswax at different concentrations. Most satisfactory results were obtained using glycerol at the concentration of 1 ml in 25 ml of potato starch suspension and 1ml garlic extract. The film has desirable anti- microbial properties due to the presence of garlic extract that delayed the spoilage of bread that was kept inside it for studying the shelf-life extension properties of the film. The biodegradation studies on the film were also conducted. The film was translucent, smooth, had good flexibility and strength. From the observations it can be concluded that the resultant film was overall acceptable and has a potential to be utilized in the commercial market.

Keywords: *Biodegradable, Anti- microbial, Plasticizers, Glycerol, Potato starch, Garlic.*

1. Introduction

From the past 2 decades, the production and the use of plastics in the world have increased enormously. Nowadays, most of the synthetic polymers are produced from petrochemicals and are not biodegradable. These persistent polymers generate significant sources of environmental pollution, harming wildlife, and marine life when they are dispersed in nature. Also, they pose a significant threat to human lives because according to recent studies, Micro plastics are found present in human blood. Concerns about the environment along with client demand for excessive high-quality, environmentally pleasant, in the direction of natural products, have drawn researchers' interest to the improvement of technologies that update the consumption of fossil substances with sustainable approaches and substances from renewable sources. In the last many years, new packaging technologies have been evolved in response to this demand, and one of the discovered answers was the development of biodegradable and suitable edible films and coatings from biodegradable polymers and renewable resources.

The term "Biodegradable" is used to describe those materials which can be degraded by the enzymatic action of living organisms, such as bacteria, yeasts, fungi and the ultimate end products of the degradation process, these being CO2, H2O and biomass under aerobic conditions and hydrocarbons, methane and biomass under anaerobic conditions [7].

Biopolymers such as polysaccharides, proteins, and lipids can be used to make biodegradable and edible films. Meanwhile, starch has been regarded as the most promising material in the production of perishable films due to their costing availability, revitalization, and biodegradability. Starch is a major plant storage form of glucose. It consists of two components: Amylose and Amylopectin. In Amylose, the glucose units are 1,4-α-D-linked together in straight chains. In amylopectin, α -(1,6) linkages are present in branched chains in addition to 1,4-α-D- linkages in straight chains. The chain length of amylose varies and it is considered to have a molecular weight of 1.1 to 1.9 million. Amylose is not truly soluble in water but forms hydrated micelles. The structure of amylose contributes to the gelling characteristics of cooked and cooled starches. In amylopectin, the glucose chains are highly branched. The length of the linear unit in amylopectin is about 20- 25 glucose units. It has a molecular weight of over 10 million. It is responsible for the thickened properties of starch preparations but it does not contribute to the gel formation. Starch can be

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gelatinized, oxidized, and reduced, and may participate in the formation of hydrogen bonds, etc. The hydrophobicity of starch can be used to improve the degradation rate of some degradable hydrophobic polymers. Biopolymers, such as proteins and polysaccharides, provide the supporting matrix for most composite films, and generally offer good barrier properties to gases, with hydrocolloid components providing a selective barrier to oxygen and carbon dioxide (Guilbert 1986 Kester and Fennema 1986; Drake et al. 1987, 1991; Baldwin 1994; Wong et al. 1992; Baldwin et al. 1997).

Potato is the fifth most important staple food crop in the world, which supplies energy and some nutritionally relevant ingredients. The nutritional value of potato tubers is mainly due to the presence of essential amino acids (esp. lysine), high contents of starch and dietary fiber as well as a low concentration of fats. Potato tubers prepared with no or low- fat addition also contain important levels of bioactive compounds and antioxidants, including phenolic acids (mainly chlorogenic acid, and flavonoids, which are phytochemicals helping to reduce the risk of several diseases, such as cardiovascular diseases (CVD) via cholesterol reduction, inflammatory pathways, and cancer. Potatoes in general are an excellent source of starch, which contributes to the textural properties of many foods. Potato starch can be used in food and other industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water-holding agent. Potato starch (PS) is one of the natural polysaccharides and is widely used in the manufacturing of edible and biodegradable films.

Amylose content of potato starch varies from 23% to 34% for normal potato genotypes (Kim et al., 1995; Wiesenborn et al., 1994; Cai and Wei, 2013). Generally, amylose is used for making strong film. When the starch molecules are heated in excess water, their crystalline structure is disrupted and water molecules become linked to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonding, which causes an increase in granule swelling and solubility. During gelatinization, the starch granule swells to several times its initial size, ruptures, and simultaneously amylose leaches out and forms a three- dimensional network. This Gelatinization property of potato starch can be utilized for developing a biodegradable film using different plasticizers and garlic extract for incorporating the antimicrobial properties in it.

Glycerol, Honey and Beeswax, by acting as a plasticizer, can improve the starchy structures by altering the structure of starch cells. The addition of plasticizers is very crucial to improve film flexibility:

1. Glycerol is the most widely used plasticizer in edible film production processes, due to its durability and similarity to hydrophilic bio-polymeric packaging chains. The paper industry uses glycerol as a humidifier, plasticizer, and lubricant.
2. Honey absorbs and retains moisture, thus retarding drying. Honey was used as a plasticizer to improve elasticity of other binding media (glues, or gums).
3. Beeswax is a moderate gelling agent when compared to other natural waxes like candelilla wax, sunflower wax, or rice bran wax. This is because its chemistry is more diverse: the polyesters, polycosanols, and minor components make it unique and give it a second functionality as a plasticizer. (Puleo, S., personal communication, March 2018)

Here, Garlic is added as an antimicrobial agent. Garlic have antimicrobial properties against bacteria and fungi in both vapor and liquid forms. Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit:

1. Antibacterial activity against a wide range of Gram-negative and Gram- positive bacteria, including multidrug-resistant enterotoxigenic strains of Escherichia coli;
2. Antifungal activity, particularly against Candida albicans;
3. Antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and
4. Antiviral activity

The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g., alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica*. (Ankri, S. and Mirelman, D, 1999)

The use of biodegradable films has shown to be less expensive and, therefore, can be used by small local farmers and small agricultural businesses, as it uses simpler technology and does not require high investment in machinery. Therefore, the purpose of this research project was to test the ability to produce biodegradable and antimicrobial films from potato starch and garlic extract.

2. Methodology

Materials used:

Potatoes and garlic were procured from the roadside vegetable sellers of Indirapuram, Ghaziabad and Noida Sector 20 market. Dabur honey was bought from the Bigbasket app. Glycerol, Muslin Cloth, Pestle and Mortar, Hot air oven we used from Laboratory of Food Technology Department, SRCASW, University of Delhi.

Preparation of films:

1. *Isolation of Starch*

Potatoes were purchased from roadside vegetable sellers of Ghaziabad and Noida Sector 20 market (India). Potatoes were washed completely stripped and ground for the extraction of starch. Then distilled water was added to the ground potato and the extraction process was carried out by sedimentation process. (There was no browning in the potatoes, also the purpose was only to extract the starch from potatoes so there was no need for browning prevention).

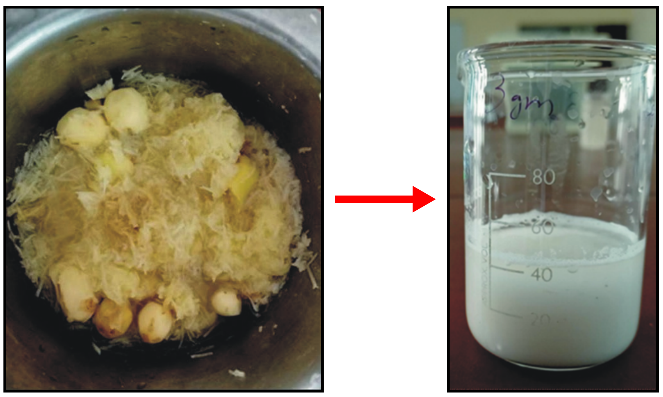


Figure 1: Isolation of starch

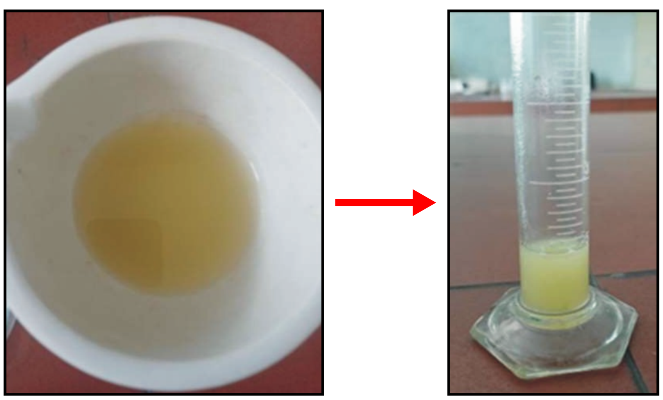


Figure 2: Garlic Extract

(a) (b) (c)

Figure 3: (a) Glycerol (b) Honey © Beeswax

Figure 4: Heating of suspension

2. *Preparation of Garlic Extract*

For the preparation of garlic extract, 7-8 g garlic was taken and grinded in the pestle and mortar by adding some distilled water.

After that, using a muslin cloth the extract got filtered in the beaker. This process was done to remove the anti-microbial extractables from garlic in water.

3. *Heating of starch suspension with addition of plasticizer and Garlic Extract*

The starch suspension was heated on the gas stove for 5 minutes to prompt starch gelatinization. Then, plasticizer (Molychem's glycerol, Dabur honey, and Anaha's beeswax pellets) was added to the above suspension and mixed uniformly. After that, garlic extract acting as an antimicrobial source was added to the above mixture and mixed for at least 2 to 3 minutes to attain some uniformity.

Note: For beeswax, first melt the pellet over a gas stove on the crucible and then use it as a plasticizer in the dispersion.

4. *Pouring and drying of film*

The gelatinized solution was poured into the petri plates and then put in the hot air oven at 85℃ for 4 hours.

5. *Removal/Peeling of the prepared film*

After complete drying of these films, they were removed from the Petri plates using scalpel and forceps. Hence, the films were prepared.

Composition of materials used in the different preparation trials of the biodegradable film:

- The different concentrations of plasticizers were taken to determine the exact concentration of plasticizer at which the film characteristics would be best. The concentration of garlic extract was kept constant at 1 ml to have its effect and to prevent the odor in the resultant films.
- Three trials of developing biodegradable film from potato starch using glycerol as a plasticizer were

FLOWCHART

Extraction of Garlic Extract (Garlic was grounded into paste and was filtered using muslin cloth)

↓

Isolation of Potato Starch and Preparation of Starch Suspension (Potato starch was suspended in 25 ml of distilled water and mixed for 2 minutes at room temperature)

↓

Heating of Starch Suspension (The suspension was heated on the gas stove for 5 minutes to prompt starch gelatinization)

↓

Addition of Plasticizer (Glycerol, honey, or beeswax (1 pellet) was added into the above dispersion as a plasticizer and was mixed uniformly)

↓

Addition of Antimicrobial source (Garlic extract was added at the required concentration and mixed for 2 to 3 minutes to get a uniform dispersion)

↓

Pouring of solution (The gelatinized solution was poured into the borosil petri plates)

↓

Drying of film (The film was put in a hot air oven at 85℃ for 4 hours)

↓

Removal of film from petri dish (After complete drying of these films, they were removed from the Petri plates using scalpel and forceps)

↓

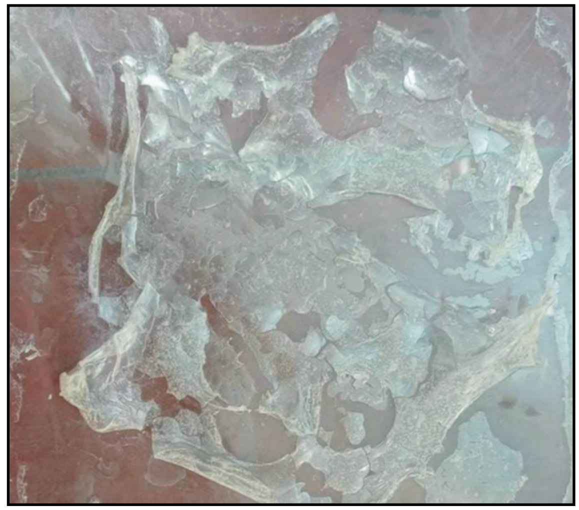
Evaluation of Film Characteristics

Figure 5: Flow Chart for preparation of film

performed. The starch suspension was 25 ml, garlic extract was 1 ml, different concentrations of glycerol were taken as 1 ml, 2 ml, and 10 ml.

- Four trials of developing biodegradable film from potato starch using honey as a plasticizer were performed. The starch suspension was 25 ml, garlic extract was 1 ml, different concentrations of honey were taken as 1 ml, 2 ml, 6 ml, and 10 ml.
- One trial of developing biodegradable film from potato starch using beeswax as a plasticizer was performed. The starch suspension was 25 ml, garlic extract was 1 ml, beeswax was 1 pellet.

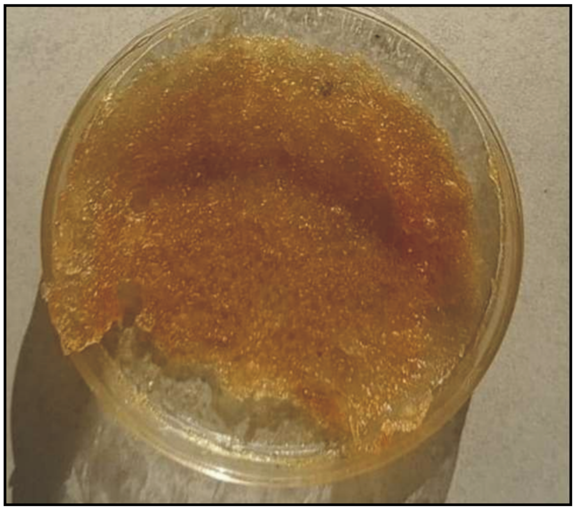
Comparative analysis of biodegradable films obtained using different plasticizers (honey, beeswax & glycerol)



Control Sample

Film using 1 ml Honey

Film using 2 ml Honey

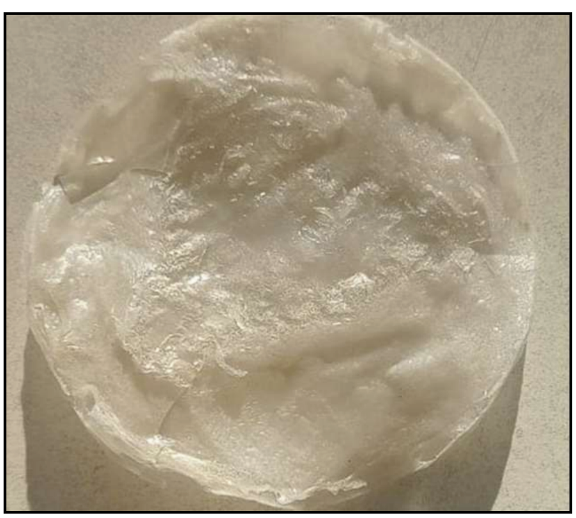


Film using 6ml honey

Film using 1 ml Glycerol

Film using 10 ml honey

Film using 2 ml glycerol



Film using Beeswax (1 Pellet)

Final Film using 150 ml starch suspension, 6ml glycerol and 6ml garlic extract

Table 1: Comparative study of films obtained using different plasticizers

Trials	Amount of starch suspension	Amount of plasticizer (Glycerol)	Amount of garlic extract	Film Characteristics
1.	25 ml	1 ml	1 ml	Flexible, transparent, elastic, good strength
2.	25 ml	2 ml	1 ml	Flexible, sticky
3.	25 ml	10 ml	1 ml	Film was wet and it took longtime to get dried, quality was not desirable

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Trial	Amount of starch suspension	Amount of plasticizer (Beeswax)	Amount of garlic extract	Film Characteristics
1.	25 ml	1 pellet	1 ml	Brittle, cloudy and opaque

Shelf- life extension properties of the film

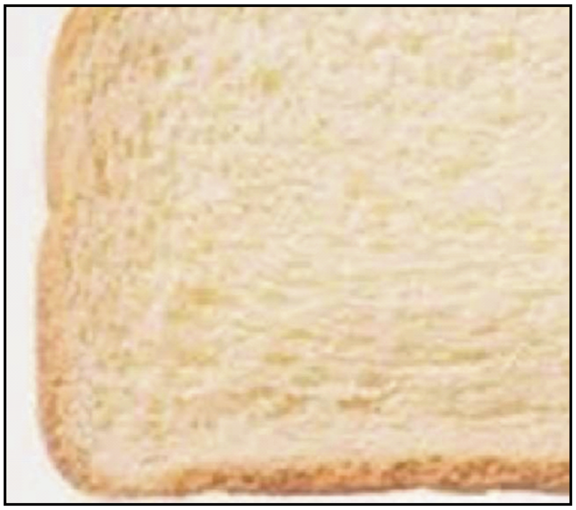
The shelf-life extension properties of the final film having 150 ml Starch suspension, 6ml Garlic extract and 6 ml glycerol were studied. A piece of Bread was used to study the shelf -life extension properties of the film. The bread sample was packed inside the film and then it is kept for a period of 7 days at the controlled conditions of temperature (above 24℃) and RH (above 70 %) to accelerate the spoilage rate. Further, the changes were noted in the bread sample.

The results were compared from a Control Bread Sample, which was kept at the similar conditions of elevated temperature and humidity without packaging it with the film. The results are as follows:

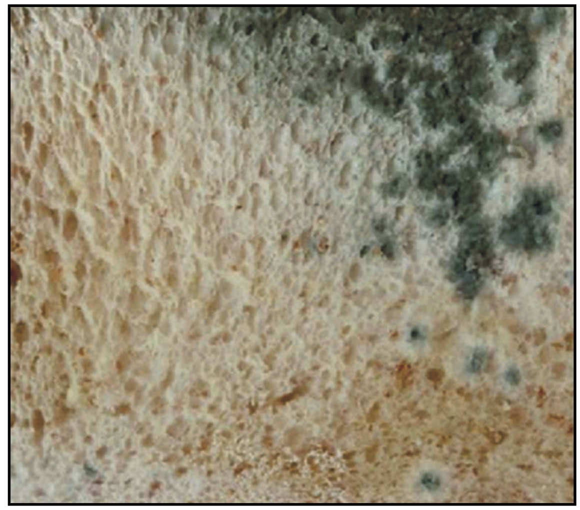
Table 2: Shelf-life Extension Properties of the Film

Bread Samples	Days	Changes in Future	Changes in Appearance (Mold Growth)
Control Sample **(Bread do not packed with the Film)**	**Day 1**	Soft	No mold growth
	Day 2	Soft	No mold growth
	Day 3	Firm	No mold growth
	Day 4	Slightly Hard	Slight whitish mold growth
	Day 5	Hard	Fuzzy whitish mold growth
	Day 6	Hard	Light green mold growth
	Day 7	Hard	Green and white mold growth
Test Sample **(Bread packed with the Film)**	**Day 1**	Soft	No mold growth
	Day 2	Soft	No mold growth
	Day 3	Soft	No mold growth
	Day 4	Soft	No mold growth
	Day 5	Soft	No mold growth
	Day 6	Soft	No mold growth
	Day 7	Firm	No mold growth

Control Sample



Day 1



Day 7

Test Sample

Day 1

Day 7

Figure 6: Shelf-Life Extension Properties of the Film

Biodegradation study of the film

The process of "Biodegradation" starts with the conversion and reduction of the long polymer molecules to shorter and shorter lengths and it undergoes oxidation (oxygen groups attach themselves to the polymer molecules). This process is triggered by heat (elevated temperatures found in landfills), UV light (a component of sunlight) and mechanical stress (e.g., wind or compaction). Oxidation causes the molecules to become hydrophilic (water attracting) and small enough to be ingestible by microorganisms, setting the stage for biodegradation to begin. Biodegradation occurs in the presence of moisture and microorganisms typically found in the environment. The microorganisms consume and degrade the matter, carbon dioxide, water, and biomass are produced and returned to nature by way of the biocycle.

The Biodegradation study on the film with 25 ml Potato starch suspension, 1ml garlic extract and 1 ml glycerol was carried out for 7 days as shown in Figure 7. It can be observed that the Potato starch-based film is getting degraded in soil in the external environmental conditions. The size of the sample is getting reduced day by day, the degradation action is due to the microorganisms present in the soil, sunlight, and water. However, when it was observed after 7 days of biodegradation, the entire potato starch-based film was completely degraded and there was no weight left. This shows that the microorganisms have been able to degrade the film in the whole part. In stages observed each day the film was getting reduced in size by biodegradation action and then further metabolized into CO_2 and water.

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

Figure 7: Biodegradation Tests

3. Conclusion

Our outcomes confirmed the ability of developing potato starch-based films incorporated with garlic extract. Garlic extract is high in antimicrobial properties and has become utilized in improving the biodegradable film's functionality. The control film made out of potato starch was brittle due to the absence of plasticizers. But the potato starch film formulated with glycerol as plasticizer via casting techniques, so very flexible. Such films were easy to deal with and they were not sticky.

The addition of plasticizers became essential to boom film properties, especially the mechanical ones. In the present study, the physical properties of the potato starch- based films prepared with different concentrations of plasticizers such as glycerol, honey and beeswax were analyzed. The best film was obtained using 1 ml glycerol with good handling characteristics,

translucent and appearance. Which was further used for shelf-life extension properties and biodegradation study. It was noted that Potato starch-based film was getting degraded in soil in the external environmental conditions that too after 7 days the entire potato starch-based film was totally degraded. On the other hand, trials with honey as a plasticizer were also done which showed that increasing the amount of honey (>2) in the films made the film flexible, thick but sticky on the other hand low amount of honey (<2) made the film brittle. Trial with Beeswax as the plasticizer in the potato starch-based film was brittle, cloudy, and opaque.

At last, the final trial with 150 ml starch suspension, 1 ml garlic extract and 6 ml glycerol gave satisfactory results. The film had good flexibility, strength, and appearance. It also delayed the spoilage of the bread and extended its shelf life and it degraded within 7 to 8 days. So, we can conclude that it can be utilized for commercial purposes in the packaging industry.

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