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Preparation of Antibacterial Smart Biofilm using Agri Waste

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Abstract: *Biofilm are fabricated by part of biomass sources, made up of bio based and are serving as very good alternatives to plastic. The study aims effective food waste valorisation by using food waste: orange peel and onion peel which are equipped with antioxidative, antibacterial properties and a PH detector compound thus making Corn flour/Orange peel/ onion peel biofilm [COO Film] an ANITIBACTERIAL- SMART EDIBLE BIOFILM.* ***.*** *Corn flour and orange peel paste were taken in different ratios; 8:2, 6:4, 4:6 and 5:5(w/w) respectively to develop the biofilm. Suspension of corn starch and orange peel was prepared with water. Followed by addition of onion peel extract and Glycerol as plasticiser. Then the mixture is heated till gelatinisation starting. Then the film was casted on glass slab and was dried in tray dryer for film preparation. Most satisfactory results were found at concentration ratio 5:5 (w/w) with 40ml of glycerol and 10ml of Onion peel extract. Also, the pH-based ability of detecting food spoilage was quite good. The film was smooth, had good flexibility and strength.*

Key Words: *Anthocyanin, Bioplastic, pH indicator, Smart packaging, Intelligent Packaging.*

1. INTRODUCTION:

Biofilm are made from biomass sources. [5] These plastics are made up of bio based, biodegradable or it can feature both the properties. Bio film can be made from different sources like starch, cellulose, chitosan, protein extracts of starch, yellow peas and from different types of vegetable and fruit waste etc.[23] These biofilm serves as very good alternatives to plastics, whose increasing load in environment has caused a lot of environmental issues. Plastics as a packaging material are being in use from a long period of time. these are very widely used for manufacturing of many different types of materials like hand bags, containers, cold drink bottles the non-biodegradable nature of plastics has been one of the major ecological issue that is being faced in recent years. The increasing load of plastics is increasing the cases of health issues faced due to accidental consumption of plastic which mainly included chocking of food pipe of many animals. Plastics many a times interact with water and form hazardous chemicals which significantly affects the quality of water. To solve this issue a large number of researches are being carried out. Many biodegradable materials are used to develop biofilm. Many bio plastics having low permeability, good tensile strength, and good young's modulus is being made from different sources like corn starch and rice starch mix, whey protein extract and many other bio unstable materials are used to develop biodegradable packaging materials. The demand of biofilm is increasing day by day mostly because of its bio-instability. The study aims effective Food waste valorisation by the use of vegetable waste: orange peel and onion peels.[14] This further helps in decreasing the load on environment of degradation of waste materials. Along with it the nature friendly biofilm is equipped with antimicrobial, antibacterial properties also which makes it preferable over other biofilms.

Orange peel biofilm; an antibacterial smart biofilm [20] is made by the combination of waste (Orange peel and onion peel). Corn flour/ Orange peel/Onion peel are used to prepare this novel biofilm. The biofilm formed was of smooth and was having compact morphology. Different ratios; 8:2, 6:4, 4:6 and 5:5(w/w) of corn-starch and orange peel paste respectively were used to make the biofilm. Best results were found at concentration ratio 5:5 (w/w) with 40ml of glycerol and 10ml of Onion peel extract. The smart aspect of detecting food spoilage was found to be best at this concentration. [11] The shelf life of the bios table biofilm is 1week.

2. LITERATURE REVIEW

Anthocyanin for active and intelligent food packaging

The use of anthocyanin (a natural dye) in the food packaging sector has been investigated because of they're their high potential properties.[18] The concept of smart packaging is basically extending the shelf life and maintaining the sensory properties of food product. Accurate analytical methods are needed to know the migration of monomers or additives from food contact materials to food. It is also important to know the number of anthocyanin that is needed to stabilize the food in order to simulate the number of anthocyanin that should migrate from the packaging into the food. For investigating the same a study was also done where the performance of combination of lysine, polyline and anthocyanin as wet colorimetric indicator of CO_2 was tested for anthocyanin stability.[19] The combination exhibits basic pH and an azure colour and upon CO_2 exposure it had showed intense purple colour. The developed indicator is stable for several weeks.

As an antioxidant material for active packaging application

A lot of studies recommended that the anthocyanin content related antioxidant activity provide fruits and the vegetables shielding against many of the degenerative and chronic diseases. The anthocyanidin and anthocyanin have shown a way more superior antioxidant activity than vitamins C and E. Anthocyanin are capable of confining free radicals through the donation of phenol hydrogen atoms which also provides anthocyanin anti-carcinogenic activity.[18] Studies have provided linear correlation between the antioxidant capability and the anthocyanin amount in blackberries, red raspberries, black raspberries and strawberries. The Anthocyanin possess the ability to act as free radical scavengers against harmful oxidants such as reactive oxygen and nitrogen species (ROS and RNS).[12] In particular, para- and Orth phenolic groups of anthocyanin are important for the formation of semiquinones and for the stabilization of one-electron oxidation products.[20] Anthocyanidins 3, 5, 7 and 3´and 4´ substituents are essential for the formation of different electronic delocalized and oxidized structures.[2, 5,4,21]

As an antimicrobial agent for active packaging application

There is very less information available about the antimicrobial activity of the pure anthocyanin. After consumption, anthocyanin are intensively metabolized in the intestines and liver. Fluorination, methylation and sulfation are the most emblematic metabolic reactions.[18] Generally, anthocyanin are active against many microbes.[19] Gram-positive bacteria usually are more vulnerable to the anthocyanin action than Gram-negative microbes. Antimicrobial activity of anthocyanin-containing fruits is because of multiple mechanisms and synergies that occur due to the presence of compounds including anthocyanin, weak organic acids, phenol acid and many more.[20]

As an indicator for intelligent packaging application

Natural dyes which are extracted from plants serves as good alternatives for use in biodegradable packing materials. Anthocyanin have also been used for making ph. sensing films. Anthocyanin extracted from purple sweet potato was used to developed a film using agar potato starch.[1] **A** polyelectrolyte complex (PEC) matrix was formed between chitosan and pectin for entrapping a bioactive compound (anthocyanin) for obtaining a useful pH indicator device.[22] A smart label for pH monitoring using bacterial cellulose (BC) nanofibers doped with anthocyanin extracted from red cabbage (Brassica oleracea) has also been made. [17] The label has diluted anthocyanin which shows a clearer response to pH variation.[24] A pH-responsive film based on chitosan and curcumin has also been developed for detecting spoilage above pH 8. Similarly, a simple indicator label using the colorimetric method for monitoring shrimp freshness has also being developed. A film was prepared using back chokeberry (Aronia Metacarpal) pomace extract along with chitosan. The immobilized dye in chitosan films showed to the pH-based colour change between pH 1 to 10.[6] Acetic acid is an important chemical reagent in food industry, it mostly functions as acidity regulator. The green label had been prepared from the extract of chitosan and purple sweet potatoes as smart materials.[3] A film prepared from artemisia chirocephalid rasch gum has responded to exposure to NH3 by showing colour change from brown to brownish red. This film could be used as smart packaging materials and gas-sensing labels.[8] A biodegradable film using cassava starch, glycerol, blueberry residue has also been prepared.[10] The colour difference produced by this indicator has been evaluated by its application in buffer solutions (pH = 4, 5, 6 and 7), simulants (saline, sucrose and protein solutions) and foodstuffs (orange juice, corn oil and chicken pieces). A chitosan based smart films using blueberry and blackberry pomace extracts has been also prepared as active agents. As pH changes from 2 to 10 the films changes from rose to blue green colour.[7] A novel film based on κ-carrageenan (Car) was also developed.[9] A stable composite films has been prepared gellant gum and purple sweet potato and the prepared film had high antioxidant activity.[22]

2. METHODOLOGY :

Raw materials used in film preparation

Orange peel of fully ripened oranges from a local vendor of east Delhi, Vasundhara Enclave was used. The orange peel was washed thoroughly with water and bruises present on them were removed. The peels were cut into small pieces and were blended very finely using high speed mixers. Onion peel of light pinkish colour was bought from a local vendor of east Delhi. Peels were thoroughly washed with water and were cut into very small pieces. The peel was blended in a high-speed mixer. Corn flour and glycerol (plasticising agent) were also used in preparing the film.

Concentrations of raw materials used in film preparation in different trials

Trials	**Amount of orange peel** (gm)	**Amount of corn starch**(gm)	**Onion peel extract** (ml)	**Glycerol** (ml)	**Water** (ml)
Trial 1	20gm	80gm	5ml	5ml	100ml
Trial 2	60gm	40gm	5ml	5ml	100ml
Trial 3	40gm	60gm	5ml	5ml	100ml
Trial 4	50gm	50gm	5ml	5ml	100ml
Trial 5	25gm	25gm	10ml	10ml	75ml
Trial 6	25gm	25gm	10ml	15ml	75ml
Trial 7	25gm	25gm	10ml	20ml	75ml
Trial 8	25gm	25gm	10ml	30ml	75ml
Trial 9	25gm	25gm	10ml	40ml	75ml

Preparation of COO film with 5:5 ratio, 10 ml onion peel extract, 40 ml glycerol

Preparation of onion peels extract:

- 10gm of onion peels were weighing.
- Mix of 50ml distilled water and weighed onion peel was heated for 5 min (colour of the water will change into red colour).

Preparation of Corn flour/ orange peel suspension:

- Take 75 ml of distilled water into a beaker.
- 25 gm of corn flour is added into it and mixed very thoroughly.
- Small quantity of the corn flour suspension is added into the orange peel mesh to make a paste out of it.
- The paste was then mixed back into the corn flour/ distilled water suspension and was mixed vigorously to make a fine suspension.

Making of Corn-starch/ Orange peel/ Onion peel film:

- Added 10 ml of cooled onion peel (10% w/v) to the COO suspension. 40m [v/v] glycerol was added into the suspension.
- The suspension was made to heat a low flame till the gelatinisation starts. Casting of the suspension is done onto a glass slab (coated with a very thin layer of vegetable oil).
- The film was kept into the dryer for 2 Hour at 60 degree Celsius for preparing a fin film out of Agri waste.

Figure 1: Raw materials used in film preparation 1. Orange peel paste 2. Onion peel 3.Corn flour

Figure 2: Onion peel extract

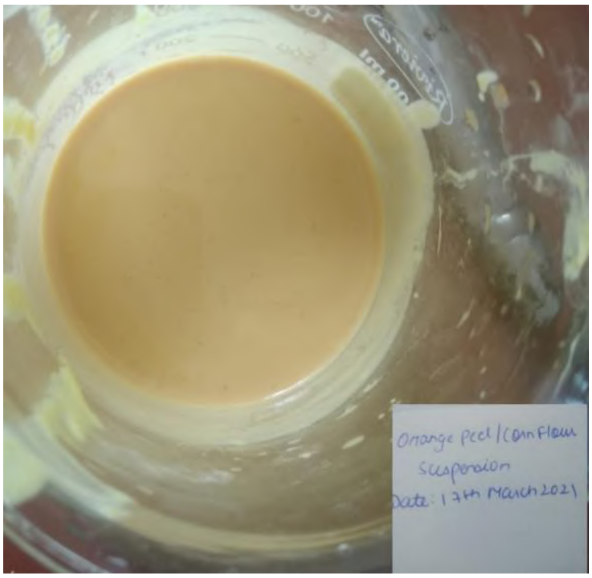

Figure 3: orange peel- corn flour suspension

Different trials for film formation

Different ratios of raw material were used while the preparation of the bio film in order to get a satisfactory result in which figure 5 Shows COO Film prepared from different amount of glycerol and 5:5 ratio of corn flour/orange peel and 10ml onion peel with different quantity of glycerol as show in figure 5.1 Film prepared with 20 ml glycerol, figure 5.2 Film prepared with 30 ml glycerol and figure 5.3 Film prepared with 40 ml glycerol

Figure 4.1: Films with 4:6 ratios of corn flour/ orange peel

Figure 4.2 Films with 5:5 ratios of corn flour/ orange peel

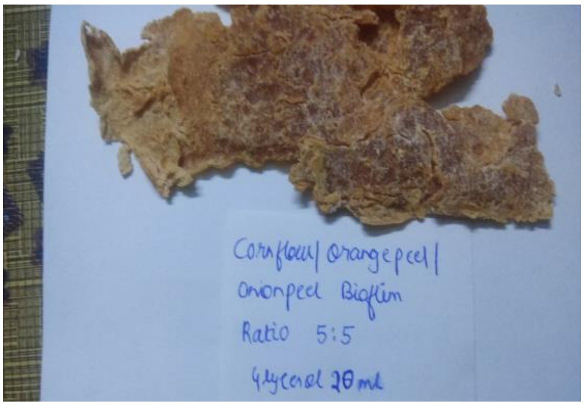

Figure 5.1.COO Film prepared with 20 ml glycerol

Figure 5.2 COO Film prepared with 30 ml glycerol

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Figure 5.3 COO Film prepared with 40 ml glycerol

3. RESULT AND DISCUSSION:

Different concentrations of raw material were used to make a satisfactory Agri-waste based smart biofilm using orange peel and onion peel. Among all the concentrations the most satisfactory result was obtained with ratio of corn starch/orange peel: 5:5 along with 10ml of onion peel extract and 40ml of glycerol. The film prepared was of 0.98mm thickness with compact morphology. It also had quite good flexibility and strength. The shelf life of the film was found to be 1 week. Most satisfactory result for pH based spoilage detecting ability was also observed at this concentration. Film showed colour change to yellow colour at basic pH and pink colour at acidic pH due the presence of onion peel extract having anthocyanin pigment whose structural variation based on number of hydroxyl groups and degree of methylation of hydroxyl group affects the colour of pigment as shown in figures 6. The property is based on PH change of the food product which can be detected by the colour change of the film due the presence of onion peel extract having anthocyanin pigment which is a flavonoid that have varied colours based on structure. The chemical form of anthocyanins is based on glycosides of flavylium salts. The structural variation of number of hydroxyl groups and degree of methylation of hydroxyl group effects the colour of pigment like excessive of hydroxyl colours leads to blue tint colour whereas high number of methyl group in the structure leads to increase in redness of pigment.

Figure 6: pH indication quality of COO Film

The will was also well equipped with ant oxidative and antibacterial properties due to the presence of orange peel having ascorbic acid and onion peel having phenols and flavonoids which as antioxidative and antibacterial components of the biofilm. The anthocyanin present in onion peels being a flavonoid have free radical scavenging property by electron delocalisation of free electron on sp2 orbital oxonium moiety or by hydrogen atom abstraction from phenolic groups. The bio plastic is also equipped with antimicrobial properties due to the presence of onion peel extract which is equipped with quercetin, phenolics and flavonoids which provide unfeasible conditions for microbial growth.[32]

4. CONCLUSION:

The aim of study of waste valorisation was successful by the development of Antibacterial-Smart bio film using Agri Waste. The film was successfully made using orange peel paste and onion peel paste as agricultural waste. Along with this two raw materials glycerol was also used as a plasticising agent and corn flour was also used as major raw material for the film formation. Nine trials were done using different concentrations of all the raw materials. Most satisfactory result was achieved with 5:5 ratio of corn flour: orange peel along with 10 ml of onion peel extract and 40 ml of glycerol. The film formed was of compact morphology with quite good strength and flexibility. The thickness of the film 0.98mm and it has a shelf life of 1 week. The film showed quite good antioxidative and antibacterial properties. The anthocyanin present in onion peels being a flavonoid has free radical scavenging property by electron preventing the biofilm from oxidation and microbial attack. The film m also had very good pH indication ability. The film showed colour change from orange to pink in acidic condition and colour change of orange to green was achieved in basic condition. The property is based on PH change of the food product which can be detected by the colour change of the film due the presence of onion peel extract having anthocyanin pigment which is a pigment that have varied colours based on structure. Colour expression of anthocyanins is influenced by its structure. Structural variations caused by a number of hydroxyl groups, the degree of methylation of these hydroxyl groups, the nature and number of sugar moieties that are being attached to the molecule. The colour variation of Anthocyanins due to the structural variations, due to differences in the number of -OH moieties in the molecule, the degree of methylation of -OH moieties, the nature and the number of the sugar moiety attached to the aglycone molecule, and the specific position of these attachments

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