Extraction, Synthesis, and Characterization of Chitosan From Fish Scales for Bioplastic Film Production

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ABSTRACT:
Plastics are widely involved in the field of packaging, construction and other form on daily basis. This is because plastics are versatile, durable and lightweight materials. Plastics are a non-biodegradable waste and it creates a drastic pollution and not able to control overall pollution in the world. Fish market which have been highly polluted by fish wastes like shells contributes huge pollution to the earth. The development of bioplastic from the fish scales has been minimized pollution from the environment by the extraction of chitosan from the fish shell. Recent studies show that the usage of bioplastics have greater impact in packaging industry. The scales of the fish scraped free of loose tissue, washed, dried in sunlight for 1 weeks and then subjected to chitosan extraction. The chitosan extracted from fish scale involves the 3 steps such as Deproteinization, Demineralization, Deacetylation and synthesis of chitosan-based bioplastic film is obtained. Degradability test was done to check whether obtained bioplastics will degrade when treated with soil microorganisms. The film is analysed in FTIR Spectroscopy to identify the interaction of ingredients in the biofilm. The cost of manufacturing the film is economical and easy to produce. Large amount of fish scales is improperly disposed at market or at home after cleaning fish, thus the fish waste is recycling and use.

Keywords: Bioplastic film, Chitosan, Fish scale, biodegradable, Food packing.

INTRODUCTION:
Biodegradable plastics can contribute to a sustainable society through the use of renewable resources and contribute to the reduction in CO_2 emissions. The biodegradable plastics offer new end-of-life management options, such as anaerobic digestion or composting, that have lower or no negative impacts on the environment (Ramadhani and Firdhausi, 2021). Bioplastics will be a new plastics generation made from raw materials such as corn, potato, or plants. They are not dangerous or hazardous and environmentally friendly raw materials. They are made in whole or in part from polymers which are derived from biological sources such as sugar cane, potato starch or cellulose from plants, straw and cotton. The following sections discuss the recent research efforts in design of packaging based on biodegradable polymers synthesized from chitosan extracted from fish scales. Unwanted off cuts from the fish processing industry creates a huge amount of waste which typically end up in a landfill which has found a new avenue as an ideal ingredient for making strong bio-plastic (Fadilla *et al.,* 2019).

Chitosan

Chitosan is a derivative of chitin, which is one of the three most important and abundant polysaccharides on the planet, together with cellulose and starch. In the manufacture of chitosan, it is divided into two stages, namely isolation of chitin in the form of deproteination, demineralization and depigmentation, then followed by the process of chitin deacetylation by reacting it with a high concentration of alkali for a long time and high temperature to become chitosan (Setha, 2019). Chitosan is a nontoxic, biocompatible, biodegradable and polyelectrolyte material that inhibits the growth of a wide variety of fungi, bacteria, and yeasts, which has allowed it to have applications in different areas such as cosmetics, agriculture, food, biomedicals, textiles (Pratiwi, 2014).

MATERIALS AND METHODS

Collection of samples

The fish scales were collected from local market in Nager coil, Tamil Nadu, and India which were transported immediately to the laboratory and stored at -20°C until analysis. The major ingredients was purchased from the local market.

Preparation of fish scales

The collected fish scales were washed and cleaned thoroughly with water to remove the impurities. Then treated using different chemical composites, i.e., 1.0 M NaCl, 0.05 M Tris HCl, and 20.0 mM EDTA for 48 hat pH 7.5. This process was intended to remove the proteins and other substances that are attached to the fish scales (Pati *et al*., 2010). Subsequently, it was cleaned and dried in a hot air oven at 70 ºC for 24 h, and powdered until very fine particles. This was sieved with a 45µm mesh and dried in a hot air oven and stored in a desiccator to avoid moisture absorbance for further processing.

Preparation of film-forming solution and film casting

Fish scale powders were the major ingredients for bioplastic film synthesis. The sample was prepared by mixing 100 ml of distilled water and 6 ml of glycerol (Thammahiwes *et al.*, 2017). The mixture was heated at 90ºC and stirred for 30 min using a magnetic stirrer on a hot plate. The film-forming solution was cast onto a rimmed silicone resin plate (50×50 mm), and air was blown over the plate for 12 h at room temperature which appeared as thin layers. These layers resulted in film formation which were manually peeled off and used for further analyses (Arfat *et al*., 2014).

Characterization of synthesized bioplastic:

Moisture content

Bioplastic samples of size 1.5 cm^2 were weighed to measure the initial weight (W_1). The samples were dried in an oven at 85C for 24 h. The samples were weighed once more to measure the final weight (W_2). The moisture content was then determined using the following formula (Sanyang *et al*., 2016):

Moisture Content(%)=(W1−W2) W1× 100

Absorption of water

Absorption of Water of the bioplastics was found out from slightly modified ASTM D570-98 method. Bioplastic samples with size 1.5 cm^2 were first dried in oven at 85 °C for 24 h to allow measuring its dry weight (W_1), followed by placing them a in beaker of 50 ml distilled water at room temperature for 24 h. After 24 h the bioplastic was obtained by filtering the water, and then its weight was measured to find its final weight (W_2). The absorption of water was found using the following formula:

Water Absorption(%)=W2− W1×100

Thickness

A handheld gauge was used for measuring the thickness of the bioplastic films. The bioplastic films produced were cut into 2c m x 2 cm dimension for testing. At random position film sample measured and value was noted.

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR Spectroscopy was used to investigate the interaction between different species and changes in chemical composition of the mixtures. The FTIR spectra of bioplastic film from fish scales were recorded in SHIMADU-8400 spectroscopy using KBR pellet method.

SEM Analysis:

The developed bioplastic film was studied at 30s of acquisition time and accelerating voltage of15 k v instrument Quanta, Thermo Fisher Scientific, USA.

RESULTS AND DISCUSSION

Synthesis of Biofilm from fish scales

The fish scales are allowed for the grinding with NaoH and allowed for the fermentation for a short period of time. After fermentation the bioplastic was synthesized by dissolving 2 g of chitosan in 25 ml of 1% acetic acid at 60^{o}C for 1 hour while stirring constantly. Then, 1g of gelatine and 1ml of glycerol was used as a plasticizer and stirred again at 60^{o} C for 15 min or until quite thick. The solution is then printed on aluminum foil, which is then baked at a temperature of 60^{o} C until the plastic solidifies in about 24 h, and resulted in the formation of film made from chitosan (Fig 1).

Fig: 1 Synthesis of bioplastic film

The current work is correlated with findings of Suneeta Kumaria and Pradip Kumar Rath (2014) who worked on extract chitin and chitosan by chemical method. Several treatments with acid and alkali were taken into consideration to determine effective concentration for yielding optimum output.

CHARACTERIZATION

Moisture content : The value for the moisture content fish scale bioplastics was noted to increase when plasticizer was added. The control has the lowest value of moisture content. Bioplastic sample with glycerol had the highest values of moisture content.

Absorption of water: The values of absorption of water were highest for the control sample.

The reason for this is that the hydroxyl group in starch has an affinity for water molecules and the gelatinisation also breaks up starch granules which lets water diffuse in (Azahari *et al*., 2011). Many studies have shown that absorption of water is directly proportional to the quantity of starch (Sujuthi and

Liew, 2016, Azahari *et al*., 2011, Aranda Garcia et al., 2015). Thus addition of plasticizer decreases absorption of water. Of the samples plasticized, the ones with glycerol had the highest absorption of water, followed by glycerol-sorbitol and then sorbitol respectively. Glycerol has a higher attraction to water molecules as compared to sorbitol molecules (Sanyang *et al*., 2016, Cerqueira *et al*., 2012).

Thickness: The determination of a film's thickness is important when estimating the barrier properties of a packaging system. The observed thicknesses of the bioplastic films obtained was ranged from 0.66–0.75 mm. The thickness could also be attributed to the presence of more solids in the bioplastic solution and the consequent formation of a viscous paste, resulting in a thicker film. A similar thickness (0.099 to 0.1599 mm) was reported by Santana *et al*. (2018) for starch-based bioplastics.

The differences in the thickness of the bioplastics value with the addition of chitosan composition variation, wherein the thickness of bioplastics will further increase with the increasing in the addition of chitosan concentration. The highest thickness of bioplastic is in the treatment E (chitosan 20%) with an average thickness of 0.106 mm and the lowest thickness was in the treatment of A (without chitosan) with an average thickness 0.089 mm. From these results, it can be concluded that the higher the concentration of chitosan, the thickness of bioplastics will increase. The thickness of bioplastics were influenced by the amount of total solids in solution as well as spacious and volume of the solution in the mold. The more total solids contained in the solution then the bio-plastics produced become thicker (Tuty Anggraini *et al.*, 2018).

FTIR Spectroscopy Analysis

Using the optimal parameters, the biofilm was prepared and its functional groups are identified using FTIR. The purpose of the identification of the functional groups is known the interaction of the ingredients during the production of the biofilms.

Here, the IR spectrum shows peaks at 2918.83, 21462.38, 1139.04, 1017.38 and 875.01 cm^{-1} as described in the following Table 1.

S. No	Peak	Characteristic absorption (cm^{-1})	Intensity	Functional groups
1.	3647.11	3500-3700	Strong, Sharp	O-H (alcohol)
2.	2918.37	2850-3000	Strong	C-H (alkane)
3.	1462.38	1400-1600	Medium, Weak, Multiple bands	C=C (alkene)
4.	1139.04	1000-1400	Strong	C-F(alkyl halide)
5.	1017.38	1000-1300	Strong	C-O (ether)
6.	875.01	1000-800	strong	O–H(alkyl halide

Table 1: FTIR Spectroscopy Analysis

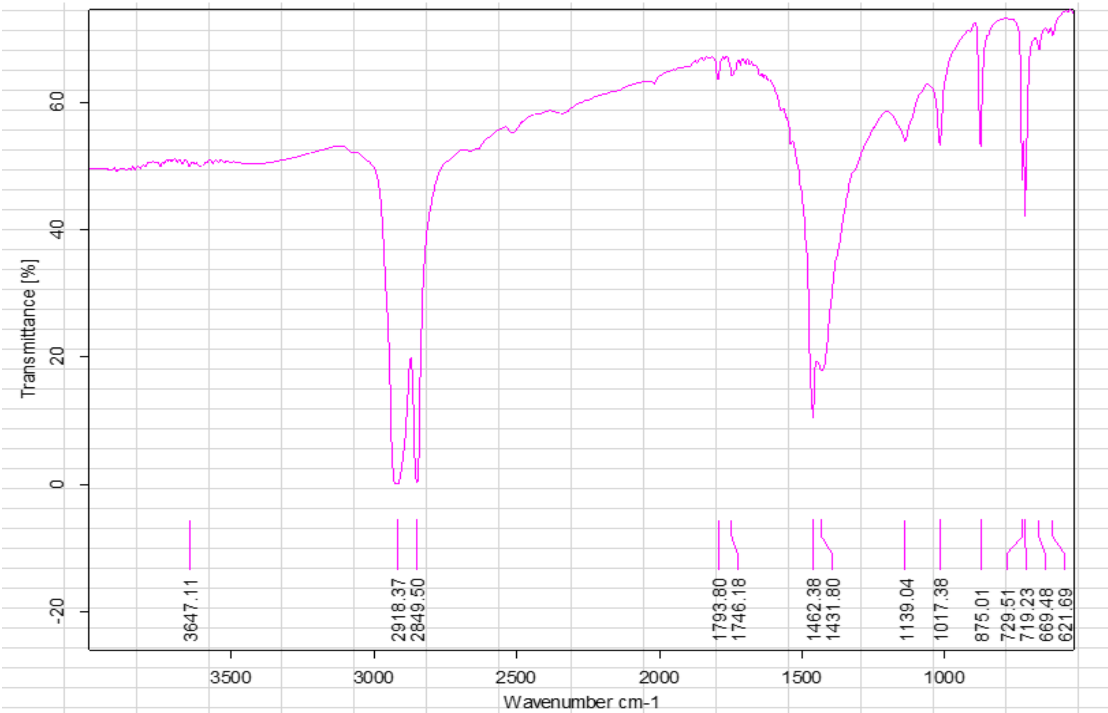

Fig .2: FTIR Analysis of the changes in the functional groups of bioplastic from chitosan

Pavia *et al*., 2001 reported functional groups and potential chemical alterations upon addition of plasticizers and fillers that were observed using the FTIR analysis. It allows for quick, authentic and efficient determination of functional groups, characteristic peaks between 2925 and 3011 cm^{-1} signifying = C-H stretching, because of the presence of starch, were noticed in all analyzed samples of both kinds of bioplastics between 2925 and 3011 cm^{-1} signifying = C-H stretching because of the presence of starch were noticed in all analyzed samples of both kinds of bioplastics.

Mutmainna *et al 2016*, reported the FTIR spectrum from the previous study shows that the bonding formation is contribution from the constituent raw material. The FTIR spectra with broad absorption peak at 3392 cm-1indicated that the presence of hydroxyl groups in the bio-composites. The C-H bonding at wave number 2917 cm^{-1}. C-O bonding was formed at 1051 cm-1, and another. C-H bonding were formed at 916 cm-1811 cm-1, and 761 cm-1,respectively. The C=C, and C-H, bonding may attribute towards strong relation in increasing the mechanical strength and the degradation of bioplastic for polymeric materials.

SEM ANANYSIS

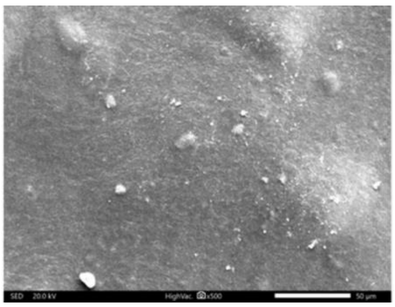

Fig 3: SEM analysis of bioplastic film synthesized from Chitosan

The bioplastic film incorporataed with chitosn showed smooth surface but some smaller voids. It displayed good incorporation of plasticizer and cross linker materials. (Vaverková *et al.,* 2012; Bahramian *et al.,* 2016; Castellani *et al.,* 2016). (Selke *et al.,* 2015)

CONCLUSION

Fish industry waste is demonstrating its great potential as a new raw material for biopolymer production in different application fields. The bioplastics are still in its infancy, and hence a great innovation would be developed by the intensification of research through the government and industrial funding. The continuing intensified research in this field would facilitate further breakthroughs and improvements.

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