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RESEARCH ARTICLE

BIO-REINFORCEMENT OF BIO-FILM BY ECO-FRIENDLY AND SIMPLE AGEING METHOD

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Abstract

In this study, Silica from agricultural waste was introduced into the network of Chitosan and the resulting changes in mechanical properties of the composite films were reported. The Chitosan bio-film was found to have improved in its mechanical properties and surface roughness after having bio-reinforced with these Mesoporous Silicon. Young's modulus, elongation at break, peak load and tensile strength were carried out on the bio-film with varying the ash contents for bio-reinforcement from 0 to 8% of the total weight of Chitosan. Mechanical properties were found to increase with increasing ash content. AFM studies were carried out on the surface of the free Chitosan film, Chitosan-black ashes composite and Chitosan-white ash composite films. Amorphous character was confirmed by XRD and FE-SEM studies were carried out for morphological study of the Chitosan-white ash composite films and Chitosan films. Maximum mechanical properties and surface roughness of the films was found with Chitosan-white ash composite.
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Keywords: Bio-reinforcement, Chitosan bio-film, simple ageing, mechanical properties and surface roughness.

Introduction

Millets are known to be the richest sources of carbohydrates and vitamins. These are food materials for most kinds of living being like birds, domestic animals and human beings. Human beings are free to consume this food, regardless of their age, sex and health discrimination. These must have been among such food materials with wide scope for consumption. Millets are available in different names and forms viz., *Panicum sumatrense, Panicum miliaceum* etc. Many of these kinds of products are mostly cultivated in hill areas. Millets are the major important food source for the rural and tribal people in Southeast Asian and African countries. Although these are consumed majorly by tribes, people in urban area also consume them in the form of millet flour especially for the growing children. T hese are smaller in size and exhibit similarity in size, shape and the rest appearance with each type of millets.

Silicon with Mesoporous nature can be helpful in many research and industrial processes. Alternative sources of Silicon, that too of biological origins, are gaining importance over the synthetic ones. This is mostly because of the environmental as well as economical reasons. There were successes with this aim of producing scalable and environmentally friendly Silicon is used agro-wastes (Batchelor L. et al.,2012). Biological sources such as rice husk ashes and bamboos are known to give Mesoporous Silicon efficiently (Della V.P et al.,2002). These products are also found to be valuable, owing to their applications (Vimal Chandra Srivasatava et al., 2006 and An Xing et al., 2013). Pure Silicon can be possibly obtained from rice husk ashes (Foletto E.L. et al., 2005). Other agro-wastes such as husk ashes of foxtail had also been successfully used to extract Silica for catalysis (Sivasubramaniam G et al., 2013), and sugarcane bagasse fly ash as the source of Zeolite in the adsorption process (Bhavna Shah et al., 2013).

Incorporation of hydrophobic polymers poly (ε-caprolactone) into the network of Chitosan had resulted in the improvement of mechanical properties of the film (Olabarrieta.I et al., 2001 and Chin-San.Wu et al., 2005). Non-polymeric inclusion into the network of Chitosan had also resulted in positive way for increasing the mechanical strength of resulting films (Khairul Anuar Mat Amin, et al., 2012). Silicon obtained from Pm has already been applied for the purpose of disassembling the synthetic polymer hydrogels (Venkatesan Srinivasan et al., 2014). In this article, Silicon from Pm husk ashes is applied for the purpose of reinforcing Chitosan bio-film and the corresponding increase in mechanical properties and surface properties are reported.

Materials and Methods

Preparation of husk ashes

Husks of Pm were collected from the local farmers. All of them were cleaned manually to remove stones and other impurities, ground well into fine pieces using mortar. They were washed with double distilled water initially and then with HCl (0.01M). Again it was washed with double distilled water to neutralize the acidic pH. They were then dried in hot air oven for 6 hours at 45-50°C and then made into ashes by the muffle furnace to the temperature range of 950°C, continuously for 12 hours. Two different husk ashes were produced as in our earlier case (Venkatesan Srinivasan et al., 2014). The one with black colour was named black ashes and the one with almost white colour was named white ashes.

Preparation of Chitosan-husk ash composites

Highly viscous Chitosan (99.9% pure) was purchased from Sigma-Aldrich, Bangalore, India. Chitosan powder is mixed with 5% acetic acid. The husk ash-Chitosan (HA-CS) composites are prepared in seven different ratios and are named accordingly. (Table.1).

The procedure was followed as such for both black and white ashes and they were used individually. The above materials were mixed on different watch glasses and allowed to spread throughout the watch glass. These were kept unperturbed in a closed glass dish and allowed for ageing in room temperature (27-28°C). The dishes were placed inside a dark case, in order to avoid the direct contact of sunlight. Silica gel was put inside to absorb moisture in the atmosphere. The films obtained after 20 days, were tested for various mechanical and surface properties (refer table 1.).

Results and Discussion

Chitosan films

Chitosan bio-films with a specific quantity of ash content as the reinforcing materials are prepared as mentioned in the experimental section. The biofilm compsoite of Chitosan and black ashes were termed Chitosan-black ash composite (refer figure 1a and that with white ash was termed Chitosan-white ash composite (refer figure 1b). The biofilms prepared without any husk ashes and therefore, purely using Chitosan as the precursor were termed free-Chitosan film (refer figure 1c). The resulting films were subjected to mechanical and surface analysis in order to study the improvements after bio-reinforcements. The mechanical properties of Chitosan films are affected by the mobility of polymer chains (Bellamy L.J. et al., 1975 and Galo cardenas et al., 2004).

FTIR spectra of free Chitosan, free black husk ashes and Chitosan composite films are shown in figures 2a, b, c and the corresponding interpretetions are given in the table 2. By the introduction of either black ashes or white husk ashes restricts the free movement of Chitosan is restricted. This is shown by the disappearance of 3550-3300 cm^{-1} broad band that was originally available with the free Chitosan, in both the kind of composite films. This also means that the intermolecular hydrogen bonding seen with free Chitosan is almost completely restricted. Further, the disappearance of –OH stretching frequency at 3928 cm^{-1} in the case of both the composite films ensures the restricted movement of the –OH entity in the Chitosan molecule by the inclusion of husk ashes of Pm. The appearance of Si-O-C stretching frequency at 1093 cm^{-1} explains for extraordinary improvement of mechanical strength of white husk ashes composite-Chitosan film.

Surface studies

Amorphous Silica is defined as a naturally occurring or synthetically produced oxide of Silicon

Table 1. Film samples

Name of the sample	Quantity of Chitosan	Quantity of reinforcements		Time of ageing in days
HA-CS 1	5.000 g	No reinforcements	0%	15 days at room temperature (27°-28°C)
HA-CS 2	4.975 g	0.025 g	0.5%	15
HA-CS 3	4.950 g	0.050 g	1.0%	15
HA-CS 4	4.925 g	0.075 g	1.5%	15
HA-CS 4	4.900 g	0.100 g	2.0%	15
HA-CS 5	4.875 g	0.125 g	2.5%	15
HA-CS 6	4.850 g	0.150 g	3.0%	15
HA-CS 7	4.825 g	0.175 g	3.5%	15

a

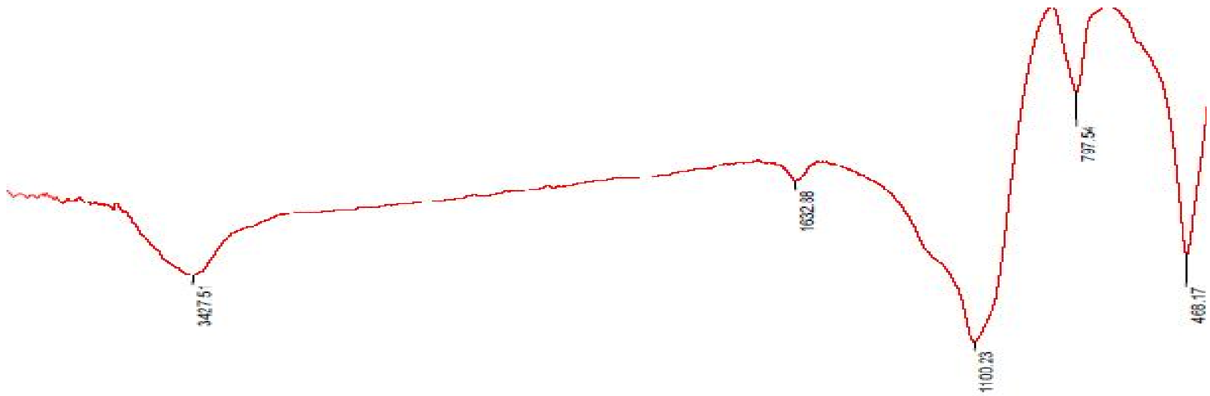
b

c

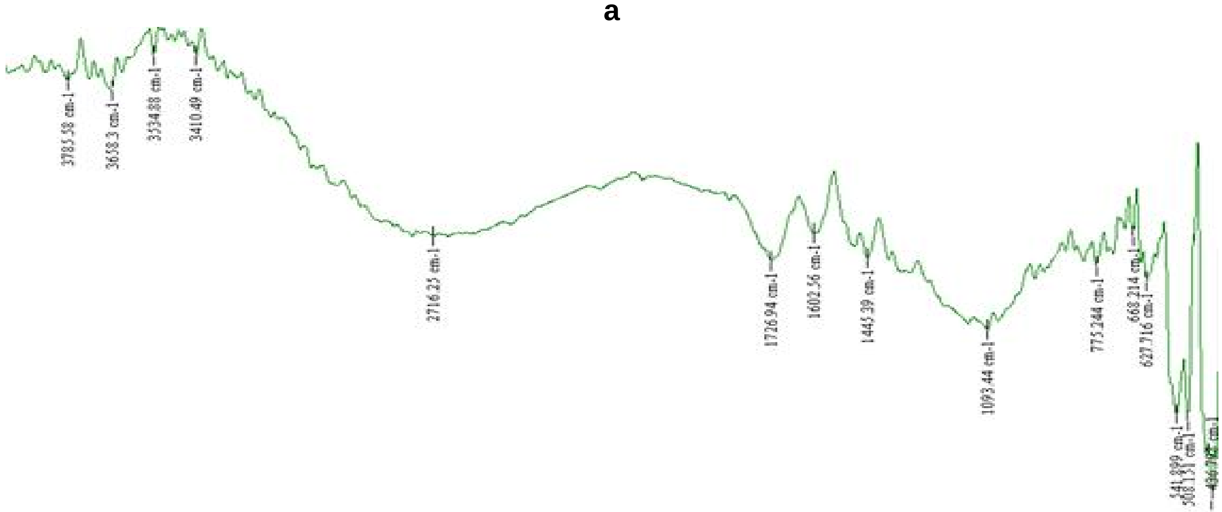
Fig.1. Photo images a) Chitosan-black ash composite film b) Chitosan-white ash composite film c)Chitosan free film

Table 2 FTIR interpretations of Chitosan and composite films

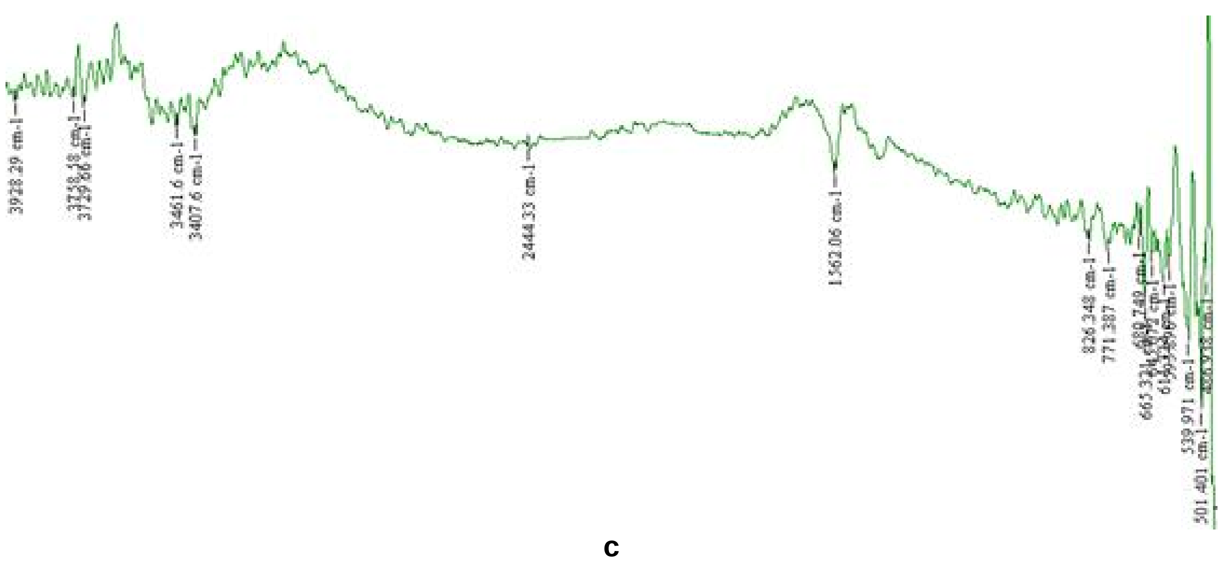
Free Chitosan film	Chitosan and black ash film	Chitosan and white ash film	Type of frequency
1726 (sharp)	-	-	-C=O stretching
3534	-	-	Si-OH stretching
3928			-O-H stretching (free)
-	-	1093 (broad band)	Si-O-C stretching
3550-3300 (broad band)	-	-	-O-H stretch (hydrogen bonded)



a



b



c

Fig.2. FTIR interaction studies of Chitosan with white ash a)free white ash b) Chitosan –black ash c)Chitosan-white ash composite

characterized by the absence of a pronounced crystalline structure and whose X-ray diffraction patterns have no sharp peaks(refer to figure 3) (Lee S.M. et al., 2010). This type of Silica may be anhydrous or have a significant water of hydration in its structure. The classification of amorphous Silica into gel or powders largely depends on the bond strengths between the ultimate colloidal particles.

Surface studies of the Chitosan bio-film were carried out by Atomic Fluorescence Microscopy (AFM) and the results are shown in fig. 4a, 4b and 4c. Surface Topography of the films suggests that the surface roughness of the resulting bio-film was found to increase with the introduction of the Pm husk ashes into the Chitosan network. Among them, the film reinforced with white husk ashes was rougher than that was reinforced with black husk ashes. This could be because of the enhanced reinforcing activity of the white husk ashes into the network the Chitosan polymers.

As the white husk ashes are prominently composing amorphous Silicon, they are provided with Silanol bonding (Zhuravlev L.T., 2010). Silanol bonding (refer figure 3) with its natural –OH ends can easily bind with the hydroxyl and amine groups of Chitosan molecule. This will enhance the surface roughness of the film (Ukrainstev.E., et al 2012). Moreover, the surface of the Silicon should have also been an influencing factor in reinforcing the biofilm (Ralph T.Yang et al., 2003). Such facilities are not available with carbon ashes. Still the black ashes composite show improved surface roughness which should be because of the little quantity of Silicon available with them and also due to filler activity of the black ashes. The semi-contact mode AFM images (Figure 4 a, b and c) revealed an increase in surface roughness in all three samples after irradiation. Before bio-reinforcement, the surface roughness of the AFM sample (Figure 4a) have been 0.71µm, which increased after 25 days of ageing with black husk ash to 0.79µm (Figure 4b) and to 0.86µm after ageing with white husk ashes. This result was evidenced by the large number of thorn-like structures found in Chitosan-white ash composite than in the other two samples. The images reflect the improvement of mechanical properties of Chitosan films after bio-reinforcement with black ashes and white ashes (Balasubramaniam Suresh et al., 2011).

FESEM analysis of free and Silica reinforced Chitosan films are shown in the figures 5 and 6 respectively. As it is evident from the morphological comparison of both free and reinforced Chitosan films, the porous appearance is the profund characteristic of the reinforced films. Strength of the materials usually improves with the increase in porous nature (Susana C.M., et al., 2010 and Hirashi yao, et al., 2007), as evidenced in the FESEM analysis.. The more the porous, the more is the strength of the film. Clearly visible porous surface of the reinforced film is an evident for the improvement of mechanical strength. However, porous surface is not observed on the free Chitosan films.

Formation of porous surface is because of the enhanced interaction between the fucntional groups of the Chitosan and the lone pair of electron on the hydroxy group of the silanol groups (HO-Si-OH). A weakly bound interaction and the availability of more space between the precursor molecules confirms the fact. Had the interaction between silanol –OH and functional group of Chitosan not taken place, the Chitosan could have not been reinforced by the husk ash of Pm.

The abundance of ‘Si’ species with the Silica reinforced films are higher and that is shown in the EDS spectrum of the corresponding films. With the free Chitosan film, such abundance is not seen showing the absence of Silicon. These facts are well described in the figures 5a and 6b.

Optical studies

Chitosan films having thickness of 0.11 mm, Chitosan-black ash composites having thickness of 1.74 mm and the Chitosan-white ash composite films having thickness of 1.73 mm were studied for their corresponding optical properties by measuring their absorbance in the range 215nm-800nm, as could be seen in the table 3. All of them exhibited absorbance in the range 245-255nm. The transmittance of all the three showed that these are less transparent (Azozano.com). This is because of the use of highly viscous Chitosan as the precursor. With the white ash compositing the % transmittance was 63% which is higher than the free Chitosan films. This fact confirms improvement of transparency of the film after having reinforced with white husk ashes. But the effect was inversed with the black ash-Chitosan composite, which is rich in carbon content. Further, hypsochromic shift is noted with inclusion of both the kind of ashes, showing conversion of C=O of free Chitosan in C-O-Si. Such a shift confirms the successful inclusion of the reinforcing material into the network of the Chitosan.

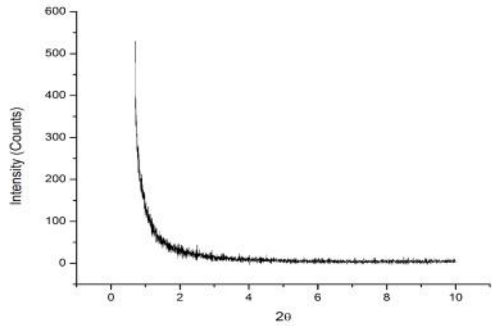


Fig.3.XRD of husk ashes

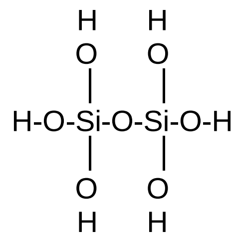
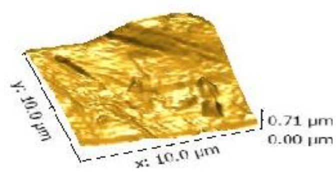
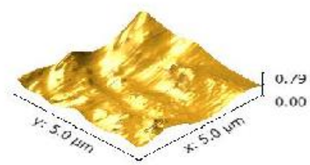
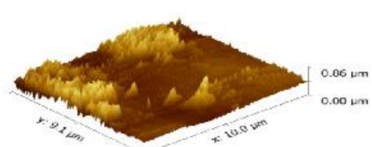


Fig.3. Structure of amorphous Silicon with silanol bonding on their surfaces





a **b**



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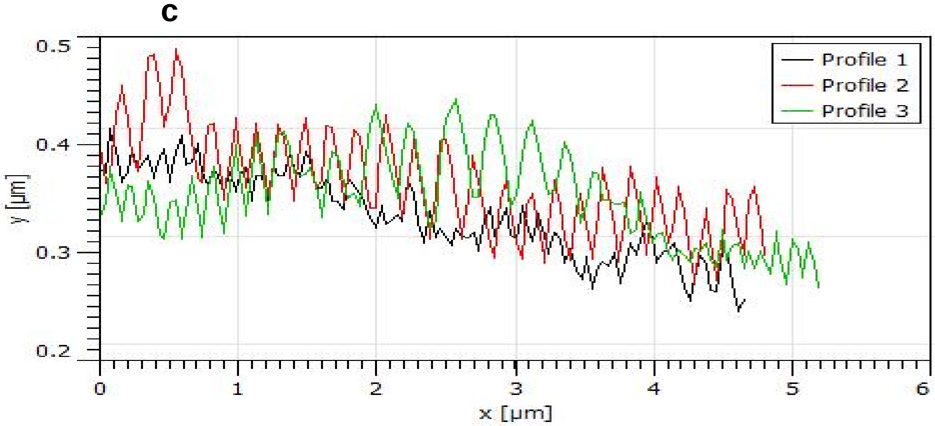

Fig. 4. AFM study of the film a)free Chitosan film b)Chitosan-black ash composite film c)Chitosan white ash composite film

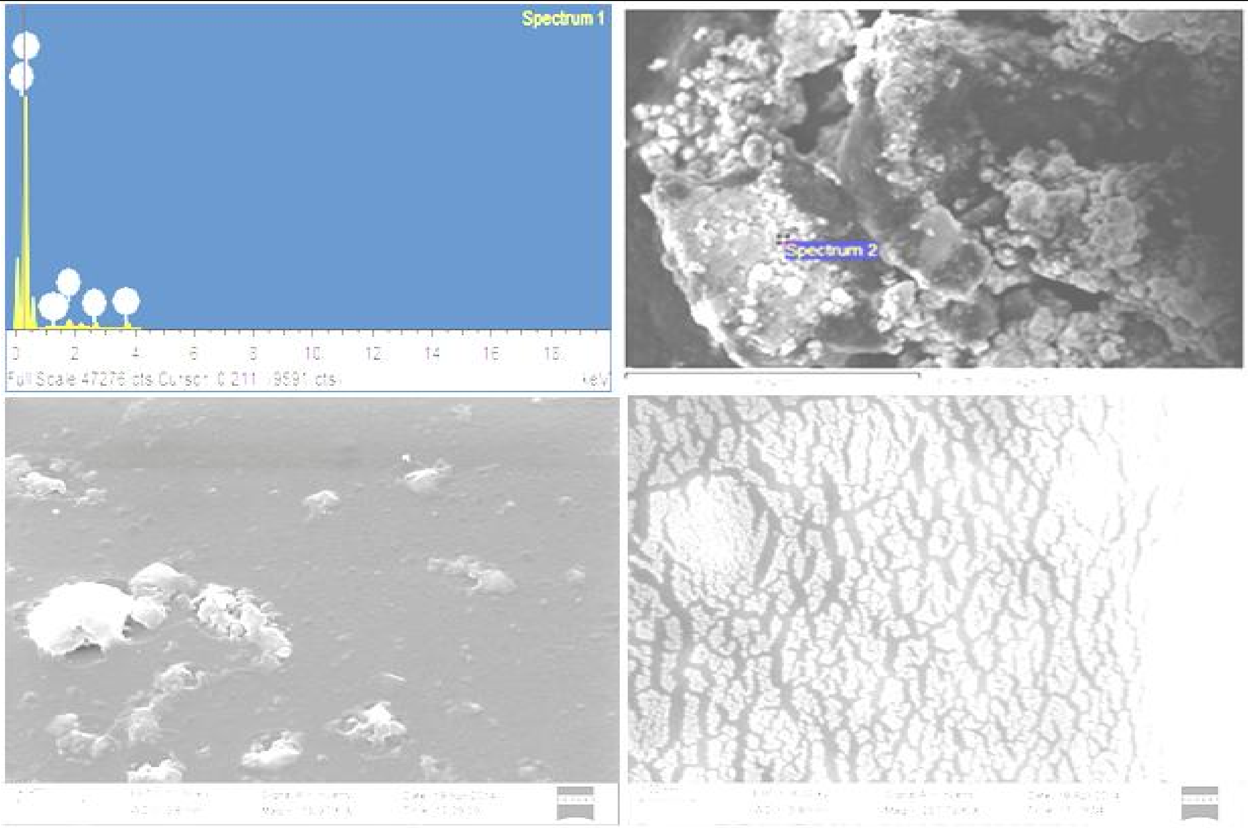

Figure.5. FESEM analysis of ash free Chitosan films a) EDS spectrum b), c) & d) surface morphology of the film

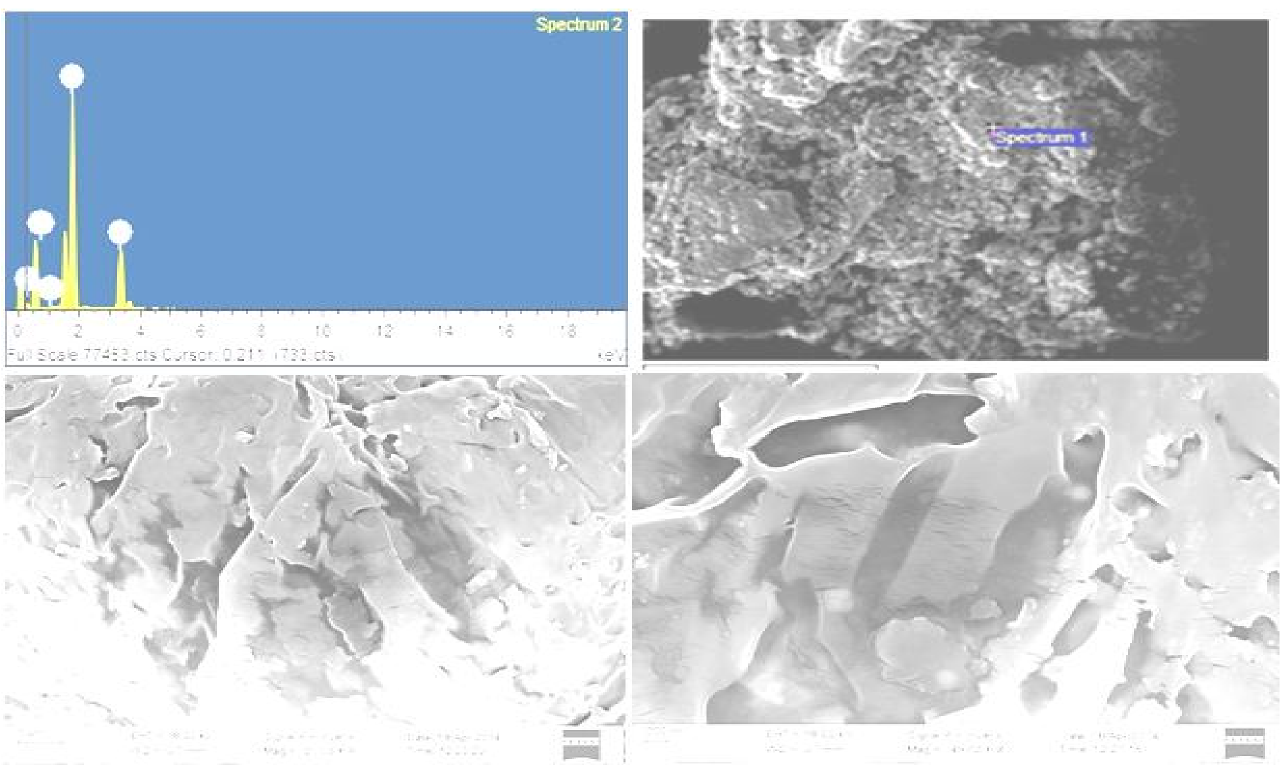

Figure.6. FESEM analysis of ash reinforced Chitosan films
a) EDS spectrum, b, c & d) surface morphology of the film

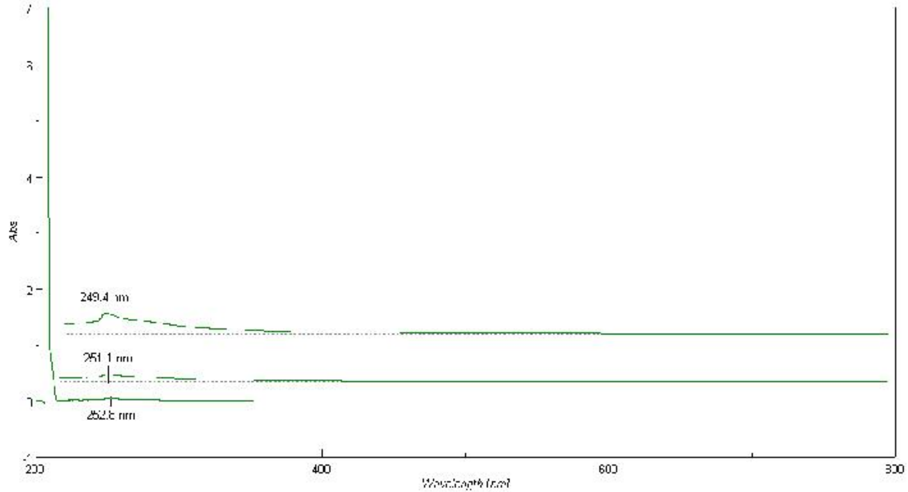

Figure.7.UV spectroscopy of Chitosan film a)Free Chitosan b)Chitosan-black ash c)Chitosan-white ash

Table 3 UV-studies

S.No	Type of film	% T	absorbance	Wavelength
1.	Free Chitosan	57.17	0.07	252.8
2.	Chitosan-black ash composite	39.82	0.4	249.4
3.	Chitosan-white ash composite	63	0.2	251.1

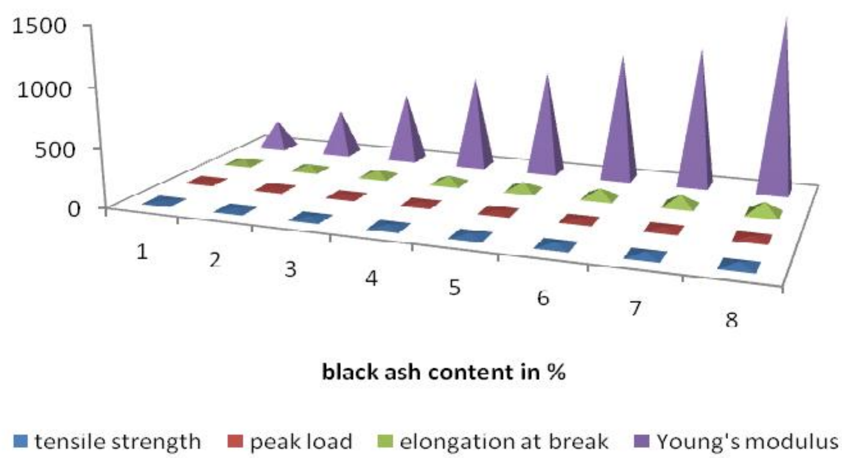

Fig.8.Variations in the mechanical properties of Chitosan film after reinforcing with black ash

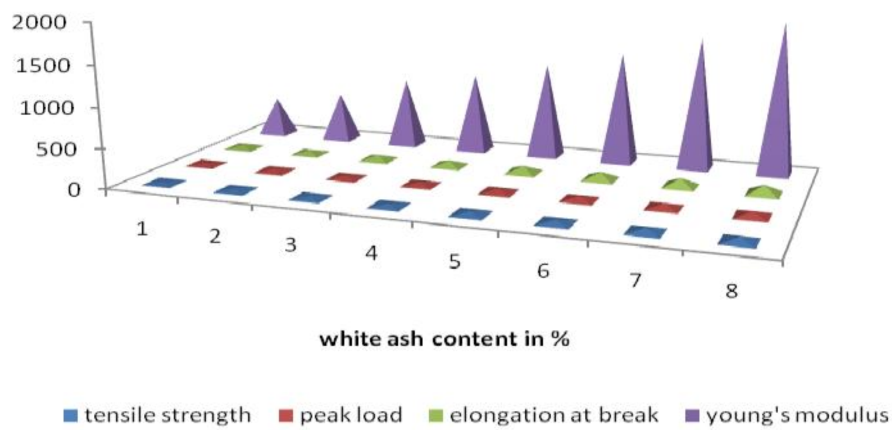

Fig.9. Variations in the mechanical properties of Chitosan film after reinforcing with white ash

Mechanical properties of Chitosan

Tensile strength of Chitosan films was improved from 32 σ/MPa to 69 σ/MPa by increasing the percentage of ash content from 0 to 8% of the total weight of the Chitosan. This gradual increase implies the improvement in mechanical properties of the film when aged with black ashes. As already discussed in the above stanza, small amount of amorphous Silicon that is available in the black ashes and the reinforcing ability of the carbon ashes are the reason behind this change. The changes that were observed with Young's modulus from 450 to 1400 E/MPa, with peak load from 8 to 21 Fb/N and with decrease in the values of elongation at break were observed, with the same range of increase in ash content, as 71 to 50 δ/% can also be acknowledged based on the above fact. These observations on the bio film clearly depict that the mechanical strength of the reinforced film had improved considerably (Jianhua Li, et al., 2013, Mohammad L., et al., 2012 and Hailong Fan et al., 2010) with black ashes.

Changes that were seen with the Chitosan-white ash composite in mechanical properties are even greater. Increase in tensile strength from 32 σ/MPa to 90 σ/MPa, the increase in peak load from 8 to 29 Fb/N and the decrease in elongation at break were 71 to 35 δ/% were noted as a consequence of ageing with white husk ash. There is also a regular variation in all these properties which confirms that the changes are purely because of the reinforcing material that was added to them. Again, the reinforcing ability of the amorphous Silicon is the key factor in improving the mechanical property of the resulting film. The amorphous Silicon content in the white ash is greater and the carbon content is almost negligible. Therefore, as expected, there is a further increment in mechanical properties when compared with the black ashes.

Conclusion

Husk ashes of Pm, being identified as the recyclable and cost effective waste bio-material, this material is used for the reinforcing purpose, to make worthy of it. Complete replacement of intermolecular hydrogen bonding by ash materials and the formation of porous surface are the reasons for improvement of mechanical strength and surface roughness of the Chitosan films. Disappearance of free –OH stretching frequency in both composites and the formation of Si-O-C bond in the white ash-Chitosan composite is the major factor behind this bio-reinforcement. This is shown by the FTIR characterizations of the respective films. AFM studies confirm improvement of the surface property of the reinforced film with the help of the thorn like appearance on the surface. Useful changes in tensile strength, peak load, elongation at break and Young's modulus confirm the role of these ashes as the bio-reinforcing materials for the Chitosan film. This work takes the advantage of reinforcing a bio-film using a naturally obtained waste husk ashes by simple and eco-friendly ageing method.

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