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Research Article

SPECTRAL, THERMAL, MORPHOLOGICAL AND MECHANICAL PROPERTIES OF SOY-PROTEIN ISOLATE MODIFIED WITH UREA

Pradosh Ranjan Das[1], Smurti Prava Das[2] Rudra Mohan Nanda[1], Harmohn Dash, P.K. Nanda and P.L. Nayak[1*]

[1]P.L. Nayak Research Foundation, Neelachal Bhavan, Cuttack, Orissa, India.
[2]Department of Chemistry, Ravenshaw University, Cuttack, Orissa, India.

***Corresponding Author: plnayak@rediffmail.com**

ABSTRACT

Soybean proteins, the by-products of oil industry, are recently considered as a petroleum polymer alternative in the manufacture of biodegradable molded products and films. In the present research program, the spectral, thermal mechanical, morphological properties and the biodegradability of the urea-modified soy protein isolate has been investigated. The water absorption increases with increase in the percentage of urea concentration. From the FTIR studies it has been ascertained that there is no bonding reaction between SPI and urea and it acts as a modifier. The thermo gravimetric analysis of the modified material has been followed using a computer analysis method, LOTUS package, developed by us for assigning the degradation mechanism. The mechanism of the degradation of the biopolymer is explained on the basis of the kinetic parameters. The tensile test results show that Young's Modulus, % elongation and tensile strength increases in the initial stages and with increase in concentration of the modifier they have a decreasing trend. The scanning electron microscopy indicates the nature of the surface morphology of the plastic. The crystallinity has been calculated by using a new computerized package method and found that mostly it represents a amorphous stage. The biodegradability of the modified SPI indicates that they degrade within reasonable time period. It is expected that this urea-modified SPI resin could be commercially used for making molded products.

Keywords: Soy proteins isolate (SPI), biodegradation and green plastics.

INTRDOCTION

The persistence of petrochemical-based plastic materials in the environment beyond their functional life has resulted in a broad range of pollution, litter, and waste disposal problems for modern society. Research to alleviate these problems includes efforts to develop plastics that degrade more rapidly in the environment. Biodegradable plastics derived from agricultural feed stocks are a new generation of materials capable of reducing the environmental impact in terms of energy consumption and green-

house effect in specific applications to perform as traditional plastics when in use and are completely biodegradable within a composting cycle through the action of living organisms when engineered to be biodegradable[1-5].

These new materials from renewable resource offer a possible alternative to traditional materials when recycling is unpractical or not economical or when environmental impact has to be minimized. Among all the agricultural products, soy protein is a good candidate which is considered to have high potential for engineering applications. In order to make soy plastics immediately competitive with petrochemical plastics, it is possible to focus on the development of soy plastics for engineering structural applications where the overall cost of producing soy plastics may be competitive with their petrochemical based plastics.

Soy protein mainly consists of the acidic amino acids of aspartic acid (asparagines) and glutamic acid (glutamine), nonpolar amino acids (glycine, alanine, valine and leucine) and basic amino acids (lysine and arginine) and less than 1% cysteine. About 90% of soy proteins are storage proteins, consisting of 35% conglycine and 52% glycinin. Soy protein has an isoelectric point at ca. pH 4.5 & at this pH , the soy protein has least net charge and thus is the most water-resistant. It is known that when the pH drops from 6 to 4.5, water absorption of the plastics decreases from ca. 80% to ca. 30% after 25 hours submersion in water at 25^{O} C[6-13].

Soy protein possesses many side reactive groups such as –NH_2, -OH, and –SH which susceptible to cross-linking reactions, in addition to naturally existing disulfide cross-links. Cross-linking leads to the formation of larger aggregates accompanied by an increase in molecular weight, reduction of solubility and reduced elasticity[14]. Investigations by several authors have shown that unmodified soy proteins are highly hydrophilic and plastics made from them, are water sensitive resulting in poor mechanical properties[15, 16]. The functional properties of soy protein are highly related to its structure. Protein modification is designed to improve functional properties by tailoring protein structures through physical, chemical, and enzymatic methods. It is well known that protein modification including denaturation can improve functional properties of food proteins, such as solubility, foaming, emulsifying, gelation, and viscosity[17]. Denaturation is defined as the modification of the secondary, tertiary, and quaternary structure of protein molecule without breaking covalent bonds present in the protein molecule. Methods of denaturation of proteins include exposure to heat, acid, alkali, detergent, or organic solvents. Nitrogen and sulphur compounds like urea, thiourea, semicarbazide & thiosemicarbazide are used as denaturing agent for protein[17].

In the present research programme, we wish to report the spectral, thermal, mechanical, and morphological properties along with biodegradability of the urea-modified soy protein for various commercial applications. The thermal degradation behaviour of the modified soy-protein isolate has been monitored by TG analysis. A novel computerized method, LOTUS package, has been used for evaluating the kinetic parameters using several kinetic equations. The values of the energy of activation have been determined using this method and the degradation steps have been explained on the basis of these parameters.

2. WORK UP PROCEDURE

2.1 Materials

Soy protein isolate (SPI), with protein cotent of about 90%, was obtained from Archer Daniels, Midland (Decatur, IL, USA) as a gift sample and used for the reaction. Urea (GR), obtained from Germany (Merk), was used without further purification for protein modification.

2.2 Preparation of Modified SPI Resin

The SPI powder (at a weight ratio of 1:10 of deionised water) was added to solutions of urea [0.0, 5, 10, 20 w/w %) of SPI & 2M] while stirring. It was allowed to react for 6 hours and was allowed to stand for 24h. Then the pH of the slurry was adjusted to 4.5 by adding propionic acid drop wise, while the mixture was continuously stirred. Then it was centrifuged to remove excess water (Sorval Superspeed RC-B; 4541g, 10min) and the precipitated residue was dried for 24h in a convection oven at ≤ 50^{O}C until a moisture content of 10% was reached. The dried modified soy protein isolate (SPI) was then milled (Cyclone Sample Mill, UDY Corporation, Fort Collins, CO, US) to pass through a 35- mesh sieve.

3. RESULTS AND DISCUSSION

3.1. Moisture Content and Water Absorption

Unmodified SPI resin as well as the compression molded SPI (control) plastic sheet showed a higher percentage of moisture content. The moisture content of the modified resin samples decreased with increase in concentration of the modifier up to 10%, after which it showed an increasing trend as evident from Fig.-1. The same trend is also seen in case of water absorption. In case of the modified SPI, urea could be the main contributor to the loss of soluble material. The molecular aggregate/entanglements and the amount of urea in the molded plastics would be the major factors influencing water absorption. Protein with more entanglements would absorb less water. At 10% urea, the water absorption is minimum, whereas at 15 and 20% urea resulted in the higher water absorption.

3.2. FTIR Analysis of the Resin

The FTIR spectra of the neat SPI [Urea-0% (Fig.2.a)] and SPI modified with urea-20% (Fig.2.b) were studied. The absorption bands related to C=O stretching at 1625 cm^{-1} (amide 1), N-H bending at 1520 cm^{-1} (amide II) and C-H deformation at 1446 cm^{-1} were observed in both. The absorption band at 1230 cm^{-1} is attributed to the C-N stretching and N-H bending vibrations. The broad absorption band observed around 3271cm^{-1} were observed in both. The absorption band at 1230 cm^{-1} is attributed to the C-N stretching and N-H bending vibrations. The broad absorption band observed around 3271cm^{-1} is attributed to the free and bounded O-H and N-H groups. The O-H and N-H groups in SPI and the O-H in absorbed water are certainly able to form inter- and intra- molecular hydrogen bonding with the C-O moiety of the amino acids (peptide and carboxyl groups) in the protein structure. The characteristic C-H stretching of the CH_2 and CH_3 groups groups of saturated structures is observed amound 2926cm^{-1}. These findings are in agreement with Moharram et al[18]. There is no change in the absorption band of the neat SPI and SPI modified with urea. They absorbed almost at the same wavelength regions. Thus, the FTIR spectra clearly indicate that, urea is not a cross-linker, rather it acts as a modifier to modify some of the properties of soy protein.

3.3. Thermogravimetric Analysis

Thermal degradation pattern of the modified biopolymers were studied using thermogravimetic analyzer (TGA 7, Perkin Elmer, Norwalk, CT) in N_2 atmosphere. The temperature range for scanning was from room temperature to 800^{o}C min^{-1} increment. The thermal degradation data of the modified SPI are furnished below (Table -1). The various kinetic equations used and the method of calculation of the kinetic parameters has been described in our previous publication[4].

A perusal of the thermo grams of the urea-modified soy protein isolate could be dissected into four steps (Fig.3: a, b,c,d & e). For example , in case of sample PKN-12, the first break takes place around 114^{o}C having weight loss about 7%, the second break takes place around 242^{o}C having weight loss of about 12% the third break takes place around 364^{o}C having weight loss about 51% and the fourth break takes place around 800^{o}C having

weight loss around 78%. In case of sample PKN-14 the first break takes place around 107°C having weight loss about 8%, the second break takes place around 250°C having weight loss of about 14%, the third break takes place around 358°C having weight loss about 52% and the fourth break takes place around 800°C having weight loss around 78%.

In case of sample PKN-18 (Fig.3 e), the break of the TG-thermogram showed a different trend as it has six breaks. The first break take s place around 103°C having weight loss about 9%, the second break takes place around 177°C having weight loss of about 13%, the third break takes place around 245°C having weight loss about 24%, the fourth break takes place around 286°C having weight loss around 45%, the fifth break around 352°C with a weight loss of 60% and the sixth break takes place around 800°C with weight loss of 81%.

This can be explained by considering the complex structure of soy-protein. It is well known that the three dimensional structure of soy-protein is governed by its primary structure, i.e. the sequence of amino acids. Two kinds of covalent bonds mainly found in proteins: one is the peptide bond between the amino acid residues and the other is the disulfide bond. The other non-covalent bonds present in protein are electrostatic and hydrophobic interactions and the hydrogen bonding[19].

In case of samples PKN-01, PKN-12, PKN-14 & PKN-16, the first break around 110°C is attributed to the elimination of absorbed water and the dissociation of the quaternary structure of proteins. Further it is well known (4) that, beyond 100°C the protein denatures their subunits and promotes the formation of protein aggregates via electrostatic, hydrophobic and disulfide interchange bonding mechanisms. This has been recently substantiated by Kilara and Sharkasi (20). It is generally accepted that, hydrophobic and disulfide bonding is involved and responsible for protein-protein aggregation caused by heating to temperature above 100°C. Further during this period the electrostatic and hydrogen bonding is also affected. The second break between 100°C to 250°C is mainly due to the cleavage of the covalent bonding between the peptide bonds of amino acid residues. During this period 69% of phenylalanine and tryptophan residues and 80% of tyrosine residue are burnt. Further heating also causes three simultaneous reactions in the structure of soy protein First, the dissociation of 7S and 11S protein subunits; second, the unfolding of the subunit secondary structure and third, the re-association of denatured subunits via disulfide, hydrophobic, electrostatic and other important bonding forces. The third break between 250-360°C is probably due to cleavage of S-S, O-N and O-O linkages of the protein molecule. The fourth break between 360-800°C is attributed to complete decomposition of protein molecule forming various gases like CO, CO_2, NH_3, H_2S and other gases. Beyond 800°C only the char residue remains.

But in case of sample PKN-18, the six-step breakage of the thermogram may be due to the presence of higher concentration of urea entangled into the protein matrix, which causes greater denaturation. It is observed in samples PKN-12 and PKN-16 that, all the four steps of degradation followed B1 mechanism. But in case of PKN-14, the first step of degradation is due to H5 mechanism & the second, third & fourth steps are due to B1 mechanism. On the other hand, all the six steps of PKN-18 follow B1 mechanism

A cursory glance at Table -2 regarding the values of activation energy for various steps of degradation is very interesting. The degradation as depicted in the thermogram takes place in four steps. The value of the activation energy, in case of all the first four samples, in the first step is comparatively high indicating slow degradation process. Subsequently the activation energy decreases in the second and then increases in the third step and subsequently decreases in the final step. In the initial step the degradation is slow

because of the elimination of the entrapped moisture in the polymer matrix, In the second step, the breakage of hydrogen bonds, disulphide and other weak bonds most probably is very fast and hence the low activation energy. In the third step again the energy of activation is higher indicating the slow process. In the third step, most probably the hard peptide and other disulphide bonds break with high activation energy thereby decreasing the rate of the reaction. Hence the mechanism of degradation of the method soy-protein isolate agrees well with the predicted mechanism as evidenced from the computed values of the energy of activation.

3.4. Differential Scanning Calorimetry

The differential scanning Calorimetry of the samples were monitored in a DuPont 2100 Thermal Analyzer from room temperature to 600^{o}C at the heating rate of 10^{o}C / min. The DSC thermo grams of the SPI modified with 0%, 5%, 10% and 20% urea are furnished in Fig.4(a, b, c, and d). The Tg and Tm of the modified samples corresponding to 5%, 10% and 20% urea were found to be 150, 152, 155 & 158^{o}C, and 382, 404, 412 and 416^{o}C respectively. Hence, the Tg and Tm increase with increase in urea concentration. This might be due to the fact that with increasing urea concentration soy protein; may be converted from the native state to the more denatured state accompanied by unfolding and disrupting of the intermolecular bonding (21). The modified products can be considered as amorphous polymers that are not arranged in order crystals, but randomly strewn together in the formation of solid state (Liang et al., 1999).

3.5. Scanning Electron Microscopy

It is well known that at relatively low concentrations (5%), urea may serve as a good plasticizer, which increases the plastic strain at break (Sun et.al.2001). At very high concentrations, urea may act as good filler, which increases the plastic thickness. Nearly linear elastic deformation and brittle fracture behaviors were observed for the unmodified SPI plastics (Fig. 5 a, b, c, d). The plastics from urea-modified SPI showed great deviation from linear deformation. All plastic samples modified with different concentrations of urea displayed rough and fluctuant fracture surfaces. Both deformation behavior and fracture surface indicated that plastics from urea-modified SPI were tougher than that from the unmodified SPI. As the concentration of urea increases, the surface of the plastics become more homogeneous indicating that at higher concentration it acts as good filler.

3.6. X-RAY Diffraction Studies

The X-ray diffraction pattern of the unmodified and modified SPI exhibit very strong ad distinct peaks at 2 θ at 88, 21, 38, 44, 72, 19, 72, 73, 72, 72 (Fig6)and 88 for PKN-1, PKN-12, PKN-14,PKN-16 and PKN-18 respectively. The values of % Xcr are given in Table-4. A perusal of the Xcr values indicates that the % crystallinity varies from 15% to 23%. By increasing the modifier concentration, the % of crystallinity first increases in the initial stages and with further increase of the modifier, the % of crystallinity decreases. Further it can be ascertained that the resin remains mostly in the amorphous state without being an ordered structure.

3.7. Biodegradability Test

The biodegradability of modified SPI resin was investigated using aerobic biodegradability procedure (ASTM D 5209-92). Compression molded sampled (25.5mm dia and 3.0 mm thickness) were broken into smaller pieces and placed in a compost devised by us. Within a short period of time the samples displayed excellent growth of bacteria on the surface. The samples were taken out periodically at regular intervals of time, washed thoroughly, pressed with paper towel, dried and weighed. The data is represented in Fig. 7 taking days interval and % weight loss with time. It was found

that they degrade within reasonable time period.

4. CONCLUSION

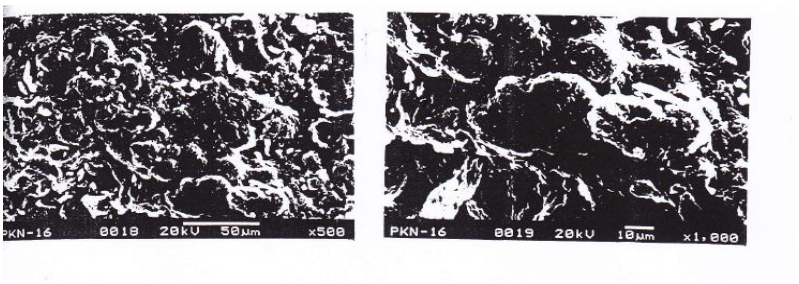
The moisture content and water absorption of the modified resin samples decreased with increase in concentration of the modifier up to 10%, after which it showed an increasing trend. Soy protein isolate is considered as a potential substitute to petrochemical plastics for the manufacture of plastics because it is agro-based, cheap, and biodegradable. Considering the global environmental problem and shortage of petroleum based plastics this is indeed a good candidate in the twenty first century for the production of environmental-friendly plastics.

Table 1: Thermal decomposition data of modified SPI with Urea in Prop ionic Acid medium

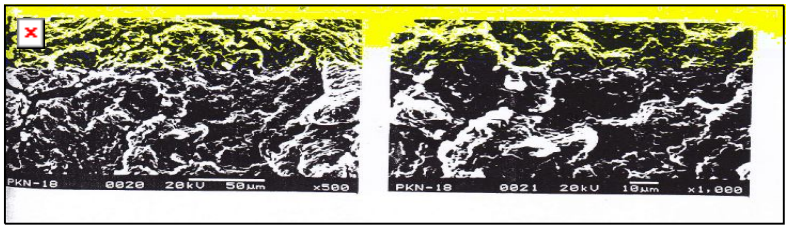
Sample	Composition	Mass loss % at various temperatures in ^{o}C							
	% of Urea 100	200	300	400	500	600	700	800	
PKN-01	00	8	12	25	55	63	68	76	83
PKN-12	05	6	10	23	60	68	71	74	77
PKN-14	10	8	11	24	63	70	73	75	78
PKN-16	20	9	14	19	58	66	73	83	91
PKN-18	2M	9	16	34	67	73	76	78	81

Table 2: Kinetic Parameters of SPI resins modified with urea

Sample	Temp Range	Model	Constant	Err. Y	R.Squared	Activation	Freequency	Slope
Code	Start-end						Energy	Factor
Steps							E(kj / mole)	
PKN01_1	38-105	B1	8.2249	0.0216	0.9863	20.10	12508.0	-1097.7
PKN01_2	106-250	B1	7.1167	0.0413	0.9509	15.56	3195.5	-849.4
PKN01_3	251-353	B1	9.4523	0.0368	0.9708	42.59	90425.3	-2325.5
PKN01_4	354-800	B1	7.3215	0.0390	0.9740	22.80	5746.4	-1244.6
PKN12_1	44-114	B1	8.0905	0.0240	0.9822	19.55	10634.5	-1067.5
PKN12_2	115-242	B1	7.3320	0.0388	0.9540	17.30	4407.2	-944.6
PKN12_3	243-364	B1	8.9910	0.0368	0.9739	37.85	50653.5	-2066.3
PKN12_4	365-800	B1	7.3058	0.0403	0.9671	21.85	5422.1	-1193.0
PKN14_1	47-107	H5	7.5245	0.0025	0.9996	16.66	5143.8	-909.4
PKN14_1	47-107	H5	7.5245	0.0025	0.9996	16.66	5143.8	-909.4
PKN14_2	108-250	B1	7.1396	0.0398	0.9531	15.80	3320.3	-862.6
PKN14_3	251-358	B1	9.3257	0.0367	0.9722	41.45	77534.2	-2263.1
PKN14_4	359-800	B1	7.2654	0.0408	0.9657	21.04	5015.4	-1148.9
PKN16_1	40-85	B1	9.1553	0.0054	0.9989	25.40	40071.5	-1387.0
PKN16_2	86-321	P1	6.6348	0.0333	0.9767	12.32	1562.7	-672.6
PKN16_3	321-705	B1	7.2434	0.0460	0.9572	20.85	4860.0	-1138.2
PKN18_1	36-103	B1	7.9884	0.0201	0.9863	18.43	9049.0	-1006.0
PKN18_2	104-177	B1	7.8698	0.0352	0.9407	20.40	8899.0	-1113.9
PKN18_3	178-245	B1	9.6178	0.0335	0.9650	38.34	96048.5	-2093.5
PKN18_4	246-286	B1	11.6895	0.0299	0.9555	62.06	1234184.7	-3388.5
PKN18_5	287-352	B1	10.8303	0.0326	0.9657	58.25	490604.5	-3180.6
PKN18_6	353-800	B1	7.2420	0.0399	0.9676	20.73	4825.2	-1131.6

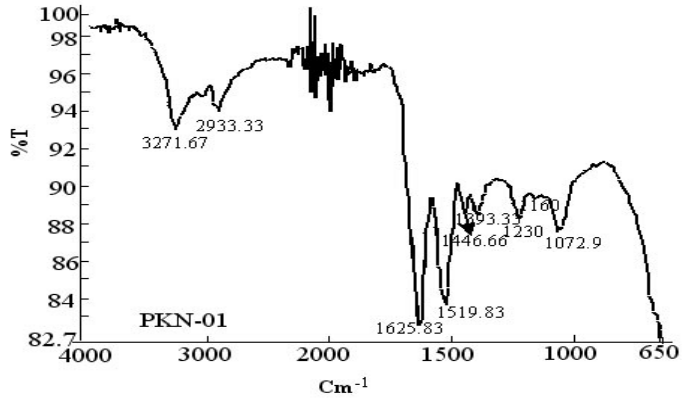


SEM of SPI with 20o/o urea



SEM of SPI modified with 2M urea

Fig.1: Moisture Content and Water Absorption of SPI/UREA



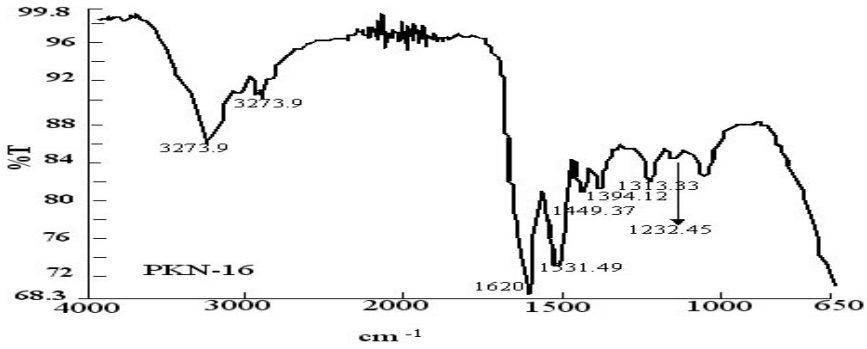


Fig. 2: FTIR Curve of SPI-UREA PKN-1(2A), PKN-16(2B)

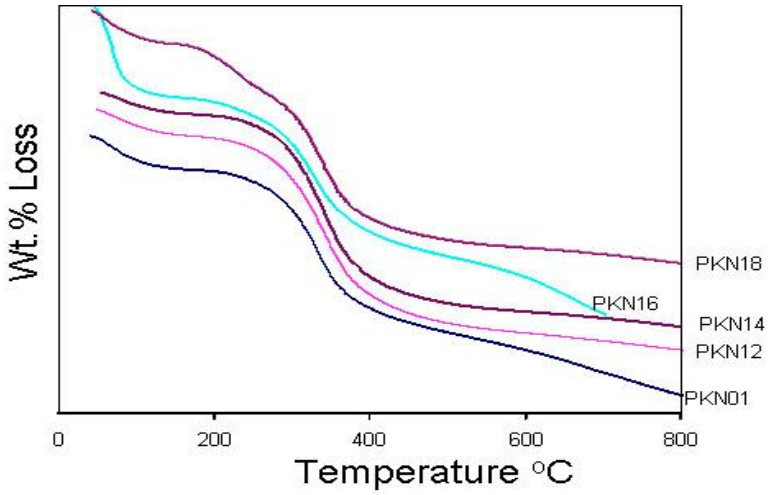


Fig . 3: TG Curve of SPI+UREA

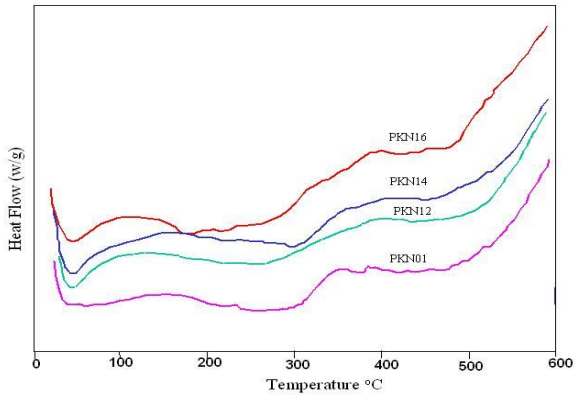


Fig. 4: DSC Curve of SPI+UREA

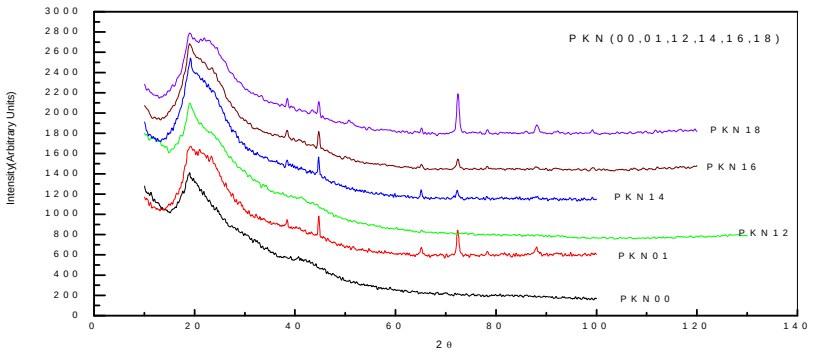
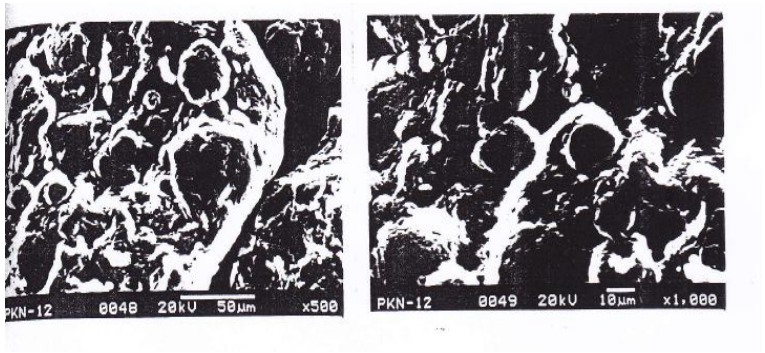


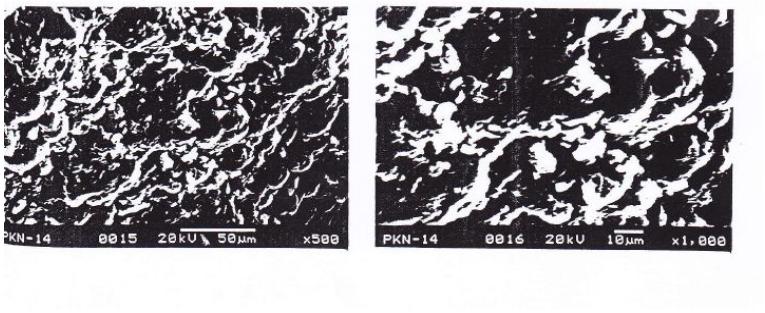
Fig. 5: Comparative XRD graph of SPI and SPI Modified with UREA (different %)

Table 3: Evaluation of crystallinity of SPI modified with Urea

Sample	2θ	Peaks	Xcr	Average Xcr	%age Xcr
PKN00	19.35	1	0.17395	0.17395	17.395
PKN01	21.85 38.75 44.75 72.40 88.15	1 2 3 4 5	0.27975 0.01385 0.11749 0.23826 0.12354	0.15457	15.457
PKN12	19.20	1	0.23314	0.23314	23.314
PKN14	19.25 44.70 65.15 72.4	1 2 3 4	0.31451 0.15351 0.17923 0.23456	0.22045	22.045
PKN16	19.25 38.45 44.70 65.15 72.55	1 2 3 4 5	0.34284 0.16495 0.21006 0.13726 0.19909	0.21084	21.084
PKN18	19.05 38.50 44.75 72.45 88.25	1 2 3 4 5	0.33532 0.14598 0.14966 0.25546 0.18236	0.21375	21.375



SPI with 5% Urea



SPI with 10% Urea

Fig. 6: SCANNING ELECTRON MICROSCOPE OF SPI/UREA

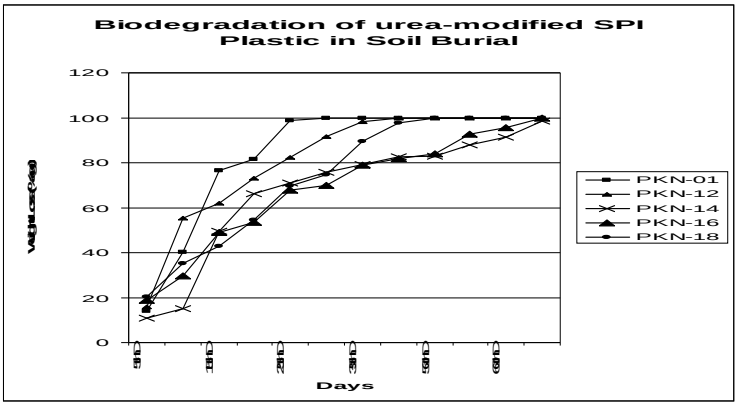
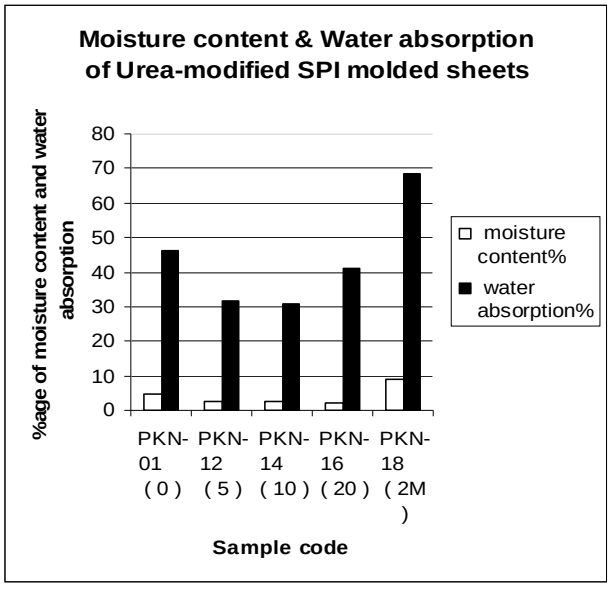


Fig. 7: Biodegradation of SPI/UREA

Table 4: Moisture content & Water Absorption (%) of urea-modified SPI sheets

Sample code	% of Urea	Moisture content %	Water absorption %
PKN- 01	(0)	4.6	46.08
PKN- 12	(5)	2.52	31.79
PKN- 14	(10)	2.38	30.62
PKN- 16	(20)	2.04	40.95
PKN- 18	(2M)	9.18	68.27



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