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Preparation and characterizations of Soya Protein isolate films Coated with Palm Stearin and Modified with Antimicrobial agent Chitosan

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Abstract:

The special protein isolate (aggregates' is 396nm in size by Laser granulometry) based film from soya bean shows good thermo stability as it started degradation beyond 50°C where as the film blended with chitosan an antimicrobial agent. Incorporated with chitosan, protein isolate film further laminated with palm stearin shows even greater thermo stability as it has started degrading from 60°C.Another wonderful observation is that films made that Soya protein isolate control films do not show much thermo stability as it starts degradation at much lower temperature like 35°C as compared to chitosan incorporated protein isolate films further laminated with palm stearin . Micro structural study reported that edible films made from special protein isolate gives a homogenous and uniform pattern, along with compact structure, upper surface shows globular arrangement. The films when incorporated with Chitosan also reveals homogeneity and compactness but at higher concentration (>50%) roughness may be observed. When the concentration of Chitosan is increased beyond 15-20%, the water vapor permeability rate and total soluble matter is decreased, thus resulting in good moisture barrier properties. The Films incorporated with Chitosan showed good inhibition against pathogenic bacteria

Keywords: special soya protein isolate**,** antibacterial agent**,** chitosan, palm stearin

Introduction:

Recently terms like biodegradable, green are used in case of packaging materials. This leads to concern of the common people regarding environmental pollution caused due to synthetic plastics. Such biodegradable plastics are usually prepared from starch (Choudhury etal 2009), cellulose (Ruan, D. et al. 2003) and protein (either plant or animal)(P.kumar etal

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2010,A.Artharn etal2010) based material. The increased interest in bio-based packaging has resulted in the development of protein-based films from soy protein, whey protein, casein, collagen, corn zein, gelatin, and wheat gluten .Among all the protein sources, soy proteins have attracted attention as a potential source for bio-based packaging materials because it has excellent film forming properties .Soya protein isolate films itself cannot meet the cost effectiveness of the packaging materials and are extremely brittle. These films are of higher water vapour permeability rate than synthetic plastics. These types of films made by casting method, extrusion techniques(P.Kumar etal 2010) and also mold pressing (Wu. etal 2001).To improve mechanical properties of such films, now a days researchers are incorporating nano materials. At nanolevel, properties of specific substance may change which is undesirable. In some recent studies, nanopores structures result from incorporation of starch nanocrystals (prepared by acid hydrolysis)into starch films(Piyada etal 2012).These nanopores create void space which may leads to weak mechanical properties. Reports on special protein isolate prepared from repetitive solubilization and crystallization process which is further prepared into film forming materials is not so far observed. Thus the objective of this work is to prepare mold pressed special soya protein isolate films incorporated with chitosan particles , and further laminated by palm stearin which is suppose to enhance the moisture barrier properties of the edible films or coating.

2. Materials and Methods:

2.1Materials:

Soya Deoiled cake ( ), glycerol, chitosan(commercial sample), palm stearin are analytical grade provided by E-Merck Pvt. Ltd, Mumbai

2.2 .*Extraction of soya special protein Isolate:*

The dehulled meal was ground in a grinder and the resulting flour was sieved by sieving machine in 35 mesh. The cowpea flour was then treated with 0.1M NaOH (w/v) solution. The resulting mixture was stirred for an hour on a magnetic stirrer. Stirred mixture was centrifuged at 6000rpm for 15 minutes. The sediment was discarded and supernatant was taken and its pH was adjusted to 4.5 which was further vacuum dried at 40°C for 8 hours. Then this resolubilisation and crystallization process was carried out for 6 times.(Tanima etal)

2.3. *Film Fabrication:*

A measured amount of soya protein isolate is taken and blended with glycerol along with chitosan (cow pea protein isolate: chitosan:: 1:1) and next the sample was then placed in between two aluminum sheets (0.2mm thick and 100mm diameter) .These sheets are placed in between two platens and a pressure of 4.5KgN is applied at 125 $^{\circ}$ C for about 5 minutes, then cooled to room temperature and the samples are cut into required dimension for further testing.(Tanima etal.)

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2.4. *Laminating With Palm Stearin:*

Palm stearin is melted in a beaker at 70°C. Then the protein films are dipped and brushed by the palm stearin in a varying proportions.

2.4. Characterization

TG/DSC curves of the films were obtained by thermo gravimetric instrument ( ). The temperature range is employed from 20 to100 ◦C with a ramp rate of 2◦C min^{-1}. The surface structures of the films were measured using a HITACHI **SEM** (S-3400N, India). The films were frozen in liquid nitrogen, and immediately snapped and then vacuum dried. The fracture surfaces (cross-section) of the films were coated with gold, and then were observed. A commercial atomic force microscope (HITACHI E-1010, Digital Instruments, India), equipped with a J scanner, was used to measure the morphologies of the films. A silicon probe (HITACHI) with a cantilever length of 125 lm and a resonant frequency of about 500 kHz was used. The scan rates were in between 0.5 and 1.0 Hz. **Film Solubility** was determined by taking film pieces of 5 mm x 5 mm, were dried at 70°C in a vacuum oven for 8 h, and then weighed to the nearest 0.0001 g for the initial dry mass. Films were immersed into 20 ml of distilled water in 50 ml screw cap tubes containing 0.01g/100 g sodium benzoate. The tubes were capped and placed in a water bath for 24 h at 25°C.The solution and film piece were poured onto (Whatman No.1) qualitative filter paper, rinsed with 10 ml distilled water, and dried at 70oC in a vacuum oven for 24 h to determine the dry mass of film. Five measurements were taken for each treatment triplicate. Total soluble matter was calculated from the initial gross mass and final dry mass using the following equation:

$$\% \text{ FS (db)} = \frac{\text{film mass before test - film mass after test}}{\text{Film mass before test.}} \times 100$$

Microbiological tests are also performed.

3. Results and Discussions:

Scanning Electron Microscopy: SEM Micrograph of the films.

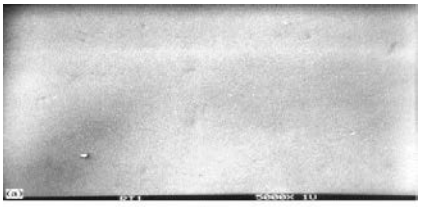

Fig1. Control film of Soya protein isolate

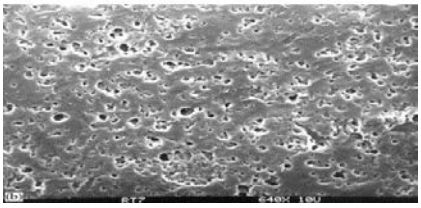

Fig2.SPI Films Incorporated with chitosan.(1%w/w)and coated by palm stearin(1%w/v)

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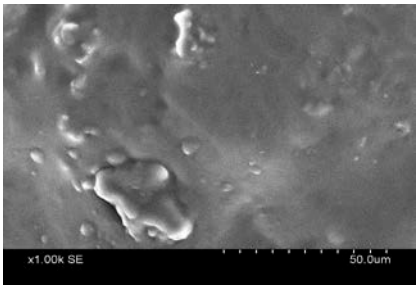

Fig3. Spi films incorporated with chitosan(2%w/w) and coated by palm stearin(2%w/v)

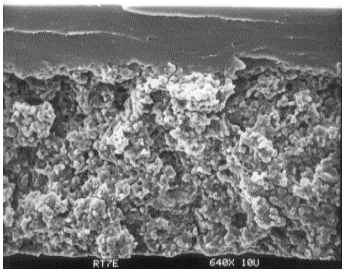

Fig.4.Spi films incorporated with chitosan(3% w/w) and coated by palm stearin(3%w/v)

Fig1 represents a uniform soya protein isolate film with smooth texture. In fig 2, film matrix shows some globular particles which is supposed to be the chitosan particle after incorporating them into control film. In fig 3 Lipid aggregates may be observed which results from palm stearin coating of the films. As the concentration of the coating is increased, aggregates of lipid particles become coarse and dense.

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DSC/TGA Graphs:

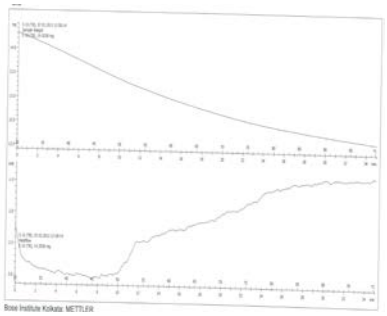

Fig.5 uncoated spi films(2% w/w chitosan)

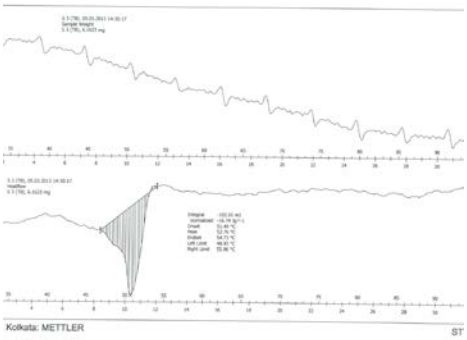

Fig6.. 2%w/v palm stearin coated spi films With 2% chitosan w/w.

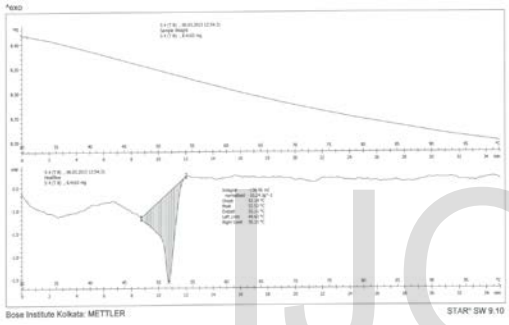

Fig7. 3% w/v palm stearin coated spi fims with 3% chitosan (w/w)

Here the above fig5. represents that uncoated spi films with chitosan started degrading beyond 30°C with a unique water loss. Wheras Fig 6 represents that the film coated with 2 % palm stearin w/v and incorporated with 2% chitosan starts degrading beyond 40°C and from Fig7 it is noted that the fiim containing 3% chitosan and coated with same concentration of palm stearin starts degradation beyond 50°C . Thus it is observed that on application of palm stearin at higher concentration results high thermal stability.

Film solubility (FS)

From visual observations and irrespective of plasticizer type and concentration, the biodegradable blend films from protein-chitosan were clearly not dispersed without visual loss of integrity after a 24 h immersion in water .Irrespective of plasticizer type, an increase in its concentrationprovided an increase in film solubility (Figure 4). Film solubility decrease from 40.19% to 22.46.The solubility values obtained in this work were relativity lower than those obtained with films based on cellulose (55-84%) or carrageen (41%) (Rhim *et al*., 1997) but higher than those obtained with fish myofibrilar proteins (Monterrey and Sorbal, 2000). An increase of film solubility with increasing plasticizer concentration could be briefly explained by

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hydrophillic plasticizers enhancing film solubility in water. The dry matter solubilized in water is likely to be constituted mainly by the plasticizer. Plasticizer solubilization in water was already observed for film based on wheat gluten or treated soy proteins or produced by transglutaminase catalytic cross-linking of whey protein (Gontard *et al*., 1992; Stuchell and Krochta, 1994). Stuchell and Krochta (1994) have pointed out an increase in the content of protein solubilized in water when the hydrophillic content increased for treated whey protein-and soy protein-based films. A decrease in the polymer network interaction density due to the plasticizer presence was thus associated with this increase in solubility properties. On the other hand, Marquei *et al*. (1995) have displayed a large decrease in the content of dry matter solubilized in water for cottonseed protein based reticulated films when glycerol content increased. It was observed that film solubility decreased when concentration of chitosan increased from 1% to 4% , SPI remaining the same along with glycerol percentage. Here the films laminated with palm stearin similar concentration to that of chitosan. Palm stearin since hydrophobic in nature may results in low film solubility from the Fig8.

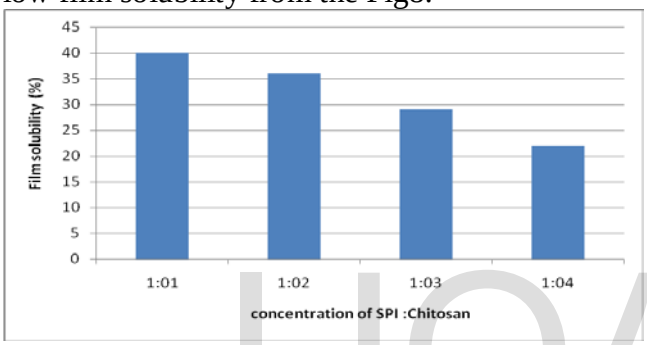

Fig8. Film solubility observed in spi films containing chitosan coated with palm sterain.

Conclusion:

The pure protein isolate films from soya bean alone cannot form stronger thermoplastics. Blends of special protein isolate with Chitosan further laminated with palm stearin may be acceptable in thermoplastics form with higher thermo stability and better moisture barrier property.

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