*Biosciences, Biotechnology Research Asia* Vol. **4**(2), 531-540 (2007)

# Mechanical properties of solution casted native and enzyme modified Soy protein films

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(Received: September 15, 2007; Accepted: November 03, 2007)

**ABSTRACT**

Solution casting is a low cost method to prepare films when compared to compression molding method. Soy protein concentrate (SPC) and soy protein isolate (SPI) have been used to prepare the films by solution casting method. Different polyols such as glycerol (G), trimethylol propane (T), polyethylene glycol (P) of different molecular weights, and enzymes of different specificity have been incorporated in soy protein to prepare the films. Tensile strength and elongation of all the prepared films were determined. TSPC films could not be prepared while TSPI films of good mechanical property can be prepared. Mechanical properties of the solution casted sample i.e GSPI-50, containing 50 % glycerol was same as that of GSPI-50 prepared by compression molding method. However, elongation and water uptake of the solution casted films were higher than that of compression molded films. Thermal stability of either solution casted/compression molded films showed no noticeable differences except in GSPI-50 (S), which showed third endothermic transition. Biodegradability of the films in the contaminated environment was also studied.

**Keywords:** Soy protein, plasticizer, solution casted, tensile strength.

## INTRODUCTION

Increasing amount of synthetic plastic wastes has led to an interest in biodegradable polymers derived from natural resources. Among all the polymers obtained from natural resources, soy protein has been considered as potential natural material. Soy protein as raw material is competitive in price with conventional petroleum based plastics and is desirable for making environmentally and biodegradable disposable products.[1] Three different types of soy proteins i.e. soy flour, soy protein concentrate and soy protein isolate, depending on the protein content, are present in the market.[2] These raw materials have been tried to prepare environmental friendly plastics. Plastics made from soy protein alone are rigid and brittle.[3] Proper plasticizers are needed to improve the processability and flexibility of soy protein plastics. Ideal plasticizers should be highly compatible with the polymers, stable at both high and low temperature. Processability and the flexibility of the soy proteins had been improved by the addition of plasticizers such as glycerol[4], ethylene glycol, triethylene glycol, polyethylene glycol, butane diols,[5,6] urea,[7] acetamide[8] to prepare soy protein plastics by compression molding method. Thermoplastic starch[9], soy protein polyester blends,[10, 11] and soy protein sheets[12] have been prepared by extrusion process. Very few literatures are available on the preparation of soy protein films by solution casting method.

Enzymatic modifications, which involve minimum side reactions and lead to limited hydrolysis of selected peptide bonds, have been

used to improve functionalities of protein. Partial enzymatic hydrolysis increases solubility, foaming and emulsifying properties. Proteolytic enzymes are generally used for such modification as they hydrolyze specific peptide bonds. Very few studies on enzymatic hydrolysis of soy protein and its effect on functional properties have been reported.[13,14,15] Structural characterization of the enzyme modified soy protein has been done by kumar et al.[16] It would be of interest to investigate the mechanical properties of soy protein films modified by enzymes such as trypsin, chymotrypsin, papain and urease having different specificity.

Soy protein concentrate and soy protein isolate has been chosen to prepare soy protein films. The focus of the present work is to prepare the soy protein films with low cost solution casting method by incorporating different polyhydric alcohols and to evaluate their mechanical properties. Best performing plasticizer was used to prepare enzyme hydrolyzed soy protein films. In addition to that biodegradability of the soy protein films has also been evaluated.

## MATERIAL AND METHODS

Soy protein concentrate (SPC) and soy protein isolate (SPI) i.e Supro 670 procured from general health store and Du Pont, India containing 73 and 92.5 % protein respectively on dry basis were used as protein source for preparing films. Trimethylol propane (TMP), glycerol, polyethylene glycol having molecular weight of 200 (PEG 200), 400 (PEG 400) and 600 (PEG 600), sodium hydroxide, glutaraldehyde (all from Qualigens, India) were used as received. Different proteolytic enzymes such as trypsin (7500 BAEE U/mg), papain (10-20 U/mg), urease (from Jackbean) (all CDH, India), chymotrypsin (40-60 U/mg), (Sigma chemicals) were used as received. Strains of *Aspergillus niger* (ATCC No. 2104) and *Cyathus bulleri* (CTCC No. 195062) (both filamentous fungi), were used for microbial growth study on the soy protein films.

### Preparation of Soy Protein Films

Soy protein films having varying amounts of different plasticizers viz glycerol (G), trimethylol propane (TMP), polyethylene glycol (PEG) were prepared by solution casting. For comparison some samples were also prepared using compression molding technique.

### Solution Casting

For the preparation of films by solution casting, 2.5 g of SPI (Supro 670) or SPC was dispersed in 25 mL of distilled water and the pH was adjusted between 9.5-10.0 using 1.0 M sodium hydroxide. Varying amounts of plasticizer such as glycerol/TMP/PEG 200/PEG 400/ or PEG 600 ranging from 12.5-75 % (w/w) with respect to SPI was added to the dispersion and mixed thoroughly using magnetic stirrer for half-an-hour. After mixing, it was poured on the glass plate kept at 50-60$^{o}$C and left undisturbed for 6-7 h and then the films were peeled off from the glass plate. Rectangular specimens prepared were used for tensile testing after conditioning for 24 h at 23±2$^{o}$C and 57 % relative humidity. The films prepared by solution casting using glycerol/TMP/PEG 200/PEG 400/PEG 600 as plasticizers have been designated as GSPI (S)/TSPI (S)/ P2SPI (S)/ P4SPI (S)/ P6SPI (S) respectively. The letter S within the parentheses represents the films obtained by solution casting process. A numerical suffix indicating the percentage of plasticizers was added to the letter designation of samples e.g. SPI film having 50 and 75 % of glycerol has been designated as GSPI-50 (S) and GSPI-75 (S) respectively. SPC film having 50 and 75 % glycerol has been designated as GSPC-50 (S) and GSPC-75 (S) respectively. SPC and SPI films in the absence of plasticizers were also prepared by solution casting technique for comparative study.

### Compression Molding

Soy protein films using 50 % (w/w) glycerol were prepared using Carver compression molding machine. For this purpose, 1.5 g of glycerol was mixed with 3.0 g of SPI powder and the powdery mixture was placed in the aluminum mold (110 x 90 x 0.25 mm). The closed mold was placed between the platens of compression molding machine which was heated to 155$^{o}$C and then a pressure of 10 ton was applied for ~7 min followed by cooling. The films obtained were designated as GSPI (C) followed by numerical suffix indicating amount of the plasticizer added. For example, SPI film obtained by using 50 % w/w of the glycerol has

been designated as GSPI-50 (C). The letter C within the parentheses represents the samples obtained by compression molding.

**Enzymatic Modification of Soy Protein**

Since enzymes act only on protein, so we can take either SPC or SPI for enzymatic modification. In this study, SPC and best performing plasticizer i.e. glycerol was chosen. For the preparation of enzymatically modified soy protein concentrate, 10 g of SPC was dispersed in 100 mL of distilled water to prepare the SPC dispersion, followed by addition of enzymes such as papain/ trypsin/ chymotrypsin/ or urease to SPC dispersion. The weight ratio of SPC:enzyme in all cases was kept at 50:1. All the substrate:enzyme dispersions prepared were hydrolyzed at 37°C for 2 h with constant stirring (200 rpm). Hydrolyzates were then inactivated by heating in boiling water bath for 5 min. 25 % of glycerol was then added to the hydrolyzates and stirred for another half-an-hour using magnetic stirrer. Enzyme hydrolyzed plasticized SPI films were prepared by solution casting using the same procedure as described above. The enzyme modified soy protein films were designated by adding a prefix C (chymotrypsin), P (papain), T (trypsin) and U (urease) to GSPC-25 (S).

**Water Absorption**

Water uptake in soy protein films, was determined according to ASTM D570-81. The specimens were pre-conditioned by drying in an air oven at 80°C for 24 h or till constant weight was obtained. The weighed samples were then submerged in distilled water at room temperature for 26 h. The specimens were removed from water and surface water was dried with filter paper before weighing. An average of three readings has been reported.

**Microbial Study**

About 3.9 g of potato dextrose agar (PDA) was dissolved in 100 mL of distilled water and the solution was autoclaved at 115°C for 20 min. The autoclaved media solution was poured on a sterile petridish under sterile conditions to prepare solid media for fungal growth (*Aspergillus niger* and *Cyathus bulleri).* In order to observe the fungal growth, soy protein films after autoclaving (115°C for 20 min) were used. Part of the autoclaved soy protein films were put on the PDA media and rest of the films were used as a nutrient source for fungal growth.. Inoculation was done by streaking the inoculums on the protein film with the help of a needle. Inoculums was the microorganism, which was made to grow on suitable nutrient media and it was prepared by growing the respective fungi on potato dextrose agar media for 4-5 days at 28°C.

**Characterization of Films**

Tensile strength of the soy protein films was determined according to ASTM D-882 using a Zwick-Z010 test machine. Rectangular specimens (11 cm long and 1.5 cm wide) were used for testing. Five specimens were tested for each formulation and the average value was reported.

TA 2100 thermal analyser having a 910 DSC module and 951 TG module was used for the thermal characterization of native and plasticized soy protein films. DSC scans were recorded in static air atmosphere at a heating rate of 10°C/min by using 5±1 mg of powdered samples. Thermal stability was determined by recording TG/DTG traces in nitrogen atmosphere (flow rate of 60 $cm^3$/ min) using powdered samples. A heating rate of 10°C /min and the sample mass of 10±1 mg was used in each experiment.

## RESULTS AND DISCUSSION

**Tensile Strength of the Soy Protein Films**

The thickness of the soy protein films was 0.24±0.05 mm. At low concentrations of the plasticizer i.e. 12.5 % or in the absence of the plasticizer, the SPI and the SPC films were very brittle and their tensile properties could not be determined. Tensile strength of all the films plasticized by different plasticizers are given in Table I. SPI films obtained by using 25 & 37.5 % of glycerol (i.e. sample GSPI-25 (S), GSPI-37.5 (S)) had higher tensile strength and low elongation as compared to the SPC films prepared using same amount of glycerol i.e. samples GSPC-25 (S), GSPC-37.5 (S). Increase in glycerol content from 25 to 75 % resulted in an increase in percentage elongation (94 % in SPC and 197 % in SPI film) and decrease in tensile strength (88 % in SPI and 75 % in SPC). Differences in the tensile strength of GSPI and GSPC samples

are due to the differences in the amount of protein present in both the types of soy protein. In materials section, it has been mentioned that SPC contains less protein content. Due to low amount of protein in SPC, there is less protein-protein interaction, which gives low tensile strength for SPC films.

Addition of 25 % and 50 % (w/w) of TMP to SPI i.e. sample TSPI-25 (S) and TSPI-50 (S) resulted in the formation of SPI films with higher tensile strength and low % elongation as compared to GSPI film having the same amount of glycerol. Higher tensile strength and low elongation may be due to crystalline nature of trimethylol propane. Interestingly, TMP does not form films with SPC by solution casting method. In addition to that no film formation was observed with PEG 200 at concentrations > 25 %, whereas, in case of TMP brittle films were obtained as concentration of TMP was > 62.5 %. Brittle films were also obtained when PEG 400 or PEG 600 were used as plasticizers in SPI or SPC. This shows that presence of cellulose in SPC prevents the formation of TMP/P2 plasticized soy protein films. The reason for this may be that in SPC most of the hydrophilic functional group of protein has already formed hydrogen bond with the –OH functional group of cellulose present in SPC. Hence, plasticizer cannot form hydrogen bond with

**Table 1. Effect of the nature of plasticizer and its concentration on the tensile properties of spc and spi films made by solution casting method.**

| Sampledesignation | Tensile strength (MPa) | Elongationat break (%) |
|---|---|---|
| GSPC-25 | 4.3±0.5 | 22.1±10 |
| GSPC-37.5 | 2.4±0.5 | 43.3±8 |
| GSPC-50 | 2.2±0.2 | 52.7±10 |
| GSPC-50* | 1.32±0.01* | 12.05±2.28* |
| GSPC-62.5 | 1.5±0.2 | 46.7±10 |
| GSPC-75 | 1.05±0.1 | 43.0±8 |
| P2SPC-25 | 6.19±0.2 | 6±1.5 |
| P2SPC-37.5 | No film formation | - |
| P4SPC-25 | No film formation | - |
| P6SPC-25 | No film formation | - |
| TSPC-25 | No film formation | - |
| TSPC-37.5 | No film formation | - |
| TSPC-50 | No film formation | - |
| TSPC-62.5 | No film formation | - |
| GSPI-25 | 6.04±0.8 | 7.73±4 |
| GSPI-37.5 | 4.74±0.4 | 10.13±2 |
| GSPI-50 | 1.78±0.06 | 24.69±5 |
| GSPI-50* | 1.77±0.18* | 4.64±0.69* |
| GSPI-62.5 | 1.24±0.11 | 26.97±1 |
| GSPI-75 | 0.68±0.06 | 23.16±5 |
| P2SPI-25 | 6.03±1.26 | 5.67±4.21 |
| P2SPI-37.5 | No film formation | - |
| P4SPI-25 | No film formation | - |
| P6SPI-25 | No film formation | - |
| TSPI-25 | 8.05±1.17 | 6.58±2.75 |
| TSPI-37.5 | 3.50±0.18 | 12.58±2.09 |
| TSPI-50 | 4.86±0.42 | 3.49±0.39 |
| TSPI-62.5 | 7.67±1.05 | 1.69±0.35 |

* represents the film prepared by compression molding method

protein due to unavailability of the hydrophilic functional groups. In case of PEG having molecular weight 400 or 600, there may be competition between the plasticizer and water (having low molecular weight) to form hydrogen bond with protein. In these cases, water forms hydrogen bond. But when the samples are being heated at 60°C, absorbed water evaporates out resulting in the formation of brittle films.

Many literatures are available on soy protein films prepared by compression molding method. Hence, for comparison only one formulation GSPC/GSPI-50 was used to prepare compression molded specimens. Films obtained using compression molding technique had significantly lower % elongation as compared to those obtained using solution casting technique (Table I). Interestingly, percentage elongation of GSPC-50 (C) was higher than that of GSPI-50 (C). This could be due to the additional plasticization effect of water being retained in the films prepared by solution casting. Thus, water expands the accessible protein conformation space leading to higher elongation in solution casted films. There is no significant difference in the tensile strength of the soy protein films prepared by either solution casting or compression molding methods.

**Effect of Enzyme Modification on Tensile Strength of Soy protein films**

The results of the tensile properties of the films obtained after enzymatic modification of SPC using 25 % (w/w) of glycerol as plasticizer are given in Table 2. Tensile strength and % elongation of all the enzyme modified samples were higher as compared to GSPC-25 except in papain modified GSPC (PGSPC-25). High tensile strength in enzyme modified samples has been observed due to selective hydrolysis of peptide bond by enzymes. Selective hydrolysis of peptide bond leads to exposure of functional groups, which may interact better (hydrogen bonding) with plasticizer giving higher mechanical properties. Chymotrypsin modified GSPC (CSPC-25) gave brittle films. Brittle films obtained using CGSPC-25 is due to the extensive hydrolysis of soy protein leading to the formation of soy protein having the molecular weight below the optimum limit for film formation. Extensive hydrolysis of soy protein by chymotrypsin is well confirmed by Kumar et al.[15]

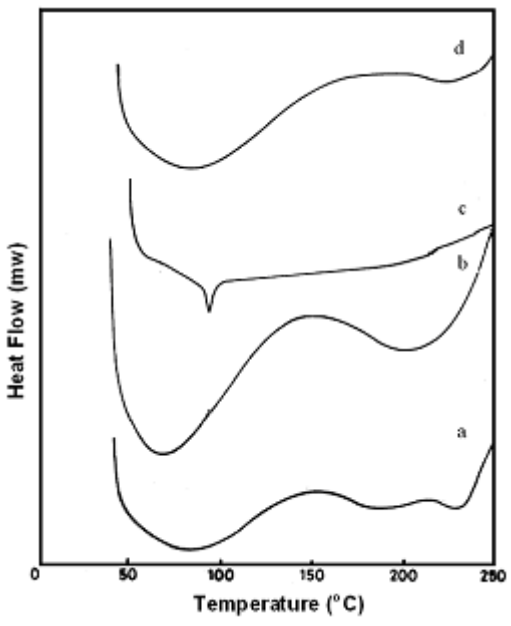



**Fig. 1: DSC scans of solution casted samples (a) GSPI-50 (S) (b) GSPC-50 (S) (c) TSPI-50 (S) (d) P2SPI-25 (S).**

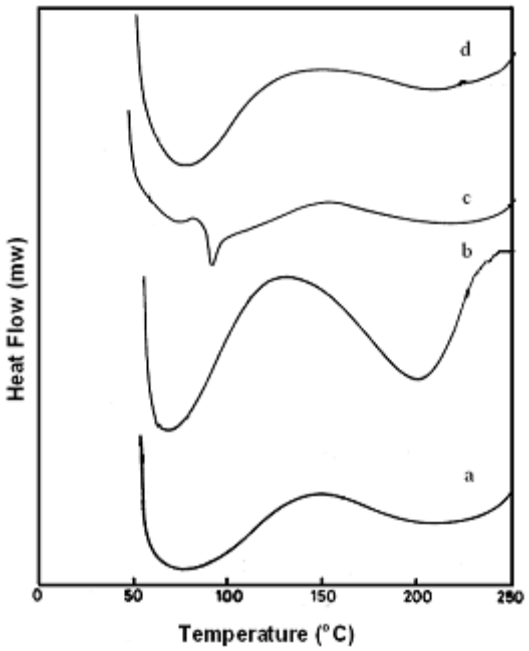



**Fig. 2: DSC scans of compression molded samples (a) GSPI-50 (C) (b) GSPC-50 (C) (c) TSPI-50 (C) (d) P2SPI-25 (C).**

**Table 2: Effect of enzymes on the tensile properties of gspc-25 prepared by solution casting method**

| Samples | Tensile strength (MPa) | Elongation (%) |
|---|---|---|
| GSPC-25 | 4.3±0.5 | 22.1±10 |
| PGSPC-25 | 3.74±0.5 | 4.03±3 |
| TGSPC-25 | 5.2±0.2 | 24.6±4 |
| UGSPC-25 | 5.4±0.5 | 43±5 |
| CGSPC-25 | No film formation | |

**Table 3: Summary of dsc analysis**

| Sample designation | First endotherm $T_p1$ (°C) | Second endotherm $T_p2$ (°C) |
|---|---|---|
| SPI (P) | 60.1 | 220.0 |
| SPC (P) | 68.0 | - |
| GSPI-50 (C) | 77.0 | 212.1 |
| GSPI-50 (S) | 83.3 | 183.3 |
| GSPC-50 (C) | 79.0 | 200.0 |
| GSPC-50 (S) | 69.5 | 196.5 |
| P2SPI-50 (C) | 66.4 | 207.7 |
| P2SPI-25 (S) | 81.5 | 223.9 |
| TSPI-50 (C) | 85.0 | 220.2 |
| TSPI-50 (S) | 83.7 | 188.1 |

**Thermal Behaviour of Soy Protein Films**

DSC scans of plasticized SPI/SPC powder and the films obtained by solution casting or compression molding are shown in Figure 1 and Figure 2. In the DSC scans (recorded at a heating rate of 10°C/min) of soy protein and its various plasticized products, a broad endothermic transition below 120°C was observed in all the samples which is due to volatilization of water. The endotherm was characterized by noting down the temperature of the endothermal peak ($T_p$) position, which was obtained by extrapolation. The results are summarised in Table III. The results of DSC scans are discussed below in terms of the nature of the plasticizer and methods of processing.

SPI showed two broad endotherms with $T_p1$ and $T_p2$ at 60.1 and 220°C, respectively, whereas SPC showed only one broad endotherm with $T_p1$ of 68.0°C (not shown in Figure). DSC scans of plasticized soy protein films prepared either by solution casting or compression molding method did not show significant changes in the endothermic peak pattern except in case of GSPI-50 (S), which showed third endothermic transition at 235°C (Figure 1). In solution casted films, water also acts as plasticizer. Water molecule offers alternative binding sites, which open the soy protein structure. This may be responsible for the appearance of the

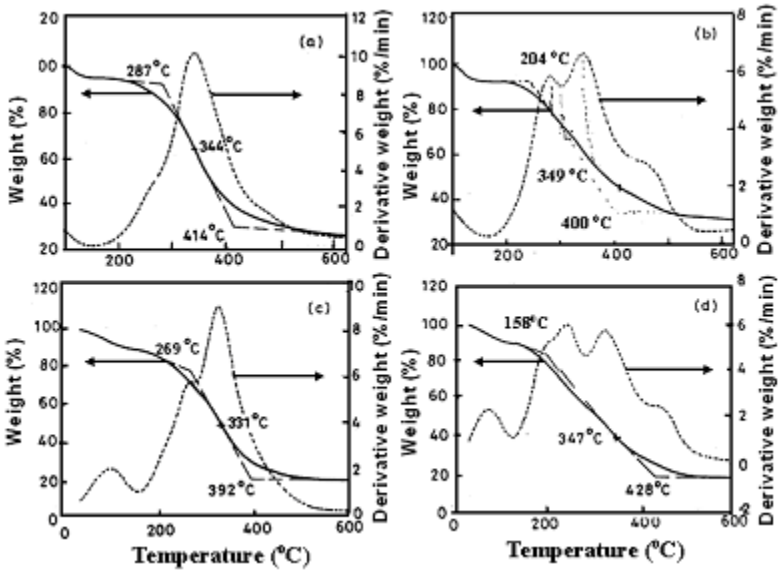



**Fig. 3: TG/DTG traces of (a) SPC (b) SPI (c) GSPI-50 (S) (d) GSPC-50 (S).**

third endothermic transition. Similar observation has already been reported in the literature.[17] All the TMP plasticized SPI films showed sharp $T_p1$ values in the range of 84±1.7°C and second broad endotherm at 188.0 and 220.2°C in case of TSPI-50 (S) and TSPI-50 (C), respectively. The sharp $T_p1$ endothermic transition in case of trimethylol plasticized soy protein samples is due to melting of plasticizer.

Typical TG/DTG traces of soy protein films in absence/presence of glycerol plasticizers are shown in Figure 3. In all the samples, multi-step mass loss behaviour was observed. Percent mass loss at different temperatures i.e 150, 200, 300 and 400°C was noted from TG/DTG traces and the results are summarized in Table IV. Plasticized samples displayed higher mass loss from room temperature to 150°C in comparison to unplasticized samples. Since the plasticized sample has the tendency to absorb more water as compared to the unplasticized sample, the mass loss was maximum in case of GSPC-50 and GSPI-50. Decrease in the amount of glycerol content resulted in the decrease in mass loss between 50-150°C. Native SPC displayed lowest mass loss (%) in the temperature range of 50-150°C, 150-200°C and 200-300°C. Higher mass loss in the plasticized sample could be due to the volatilisation of the plasticizer. The char yield at 600°C was found to be 27.5±2.5 % in case of unplasticized sample, while in plasticized sample it was found to be 16±1.5 %. Plasticizer does not leave behind any char yield so theoretically it should be 50 % of the native protein. There was a very good agreement between the char yield obtained and the calculated value. From this, it was concluded that there was no change in degradation pattern upon incorporation of glycerol.

**Water Resistance**

Figure 4 shows the water uptake soy protein films prepared by solution casting or compression molding in presence of different amount of glutaraldehyde. Solution casted soy protein films showed very high water uptake (~ 700 %) in comparison to compression molded films (~

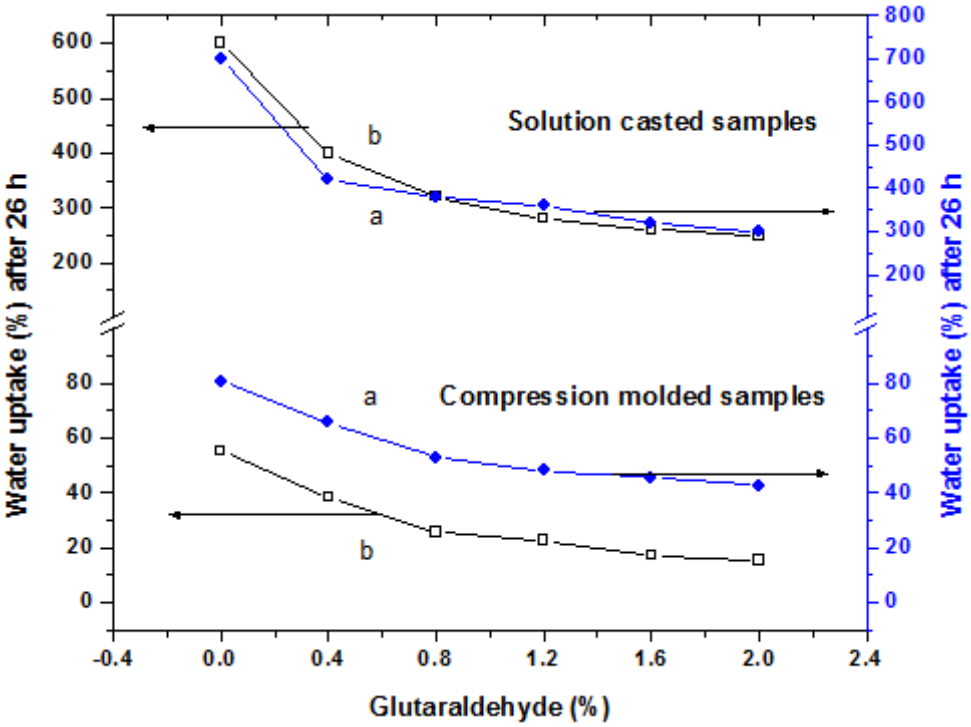


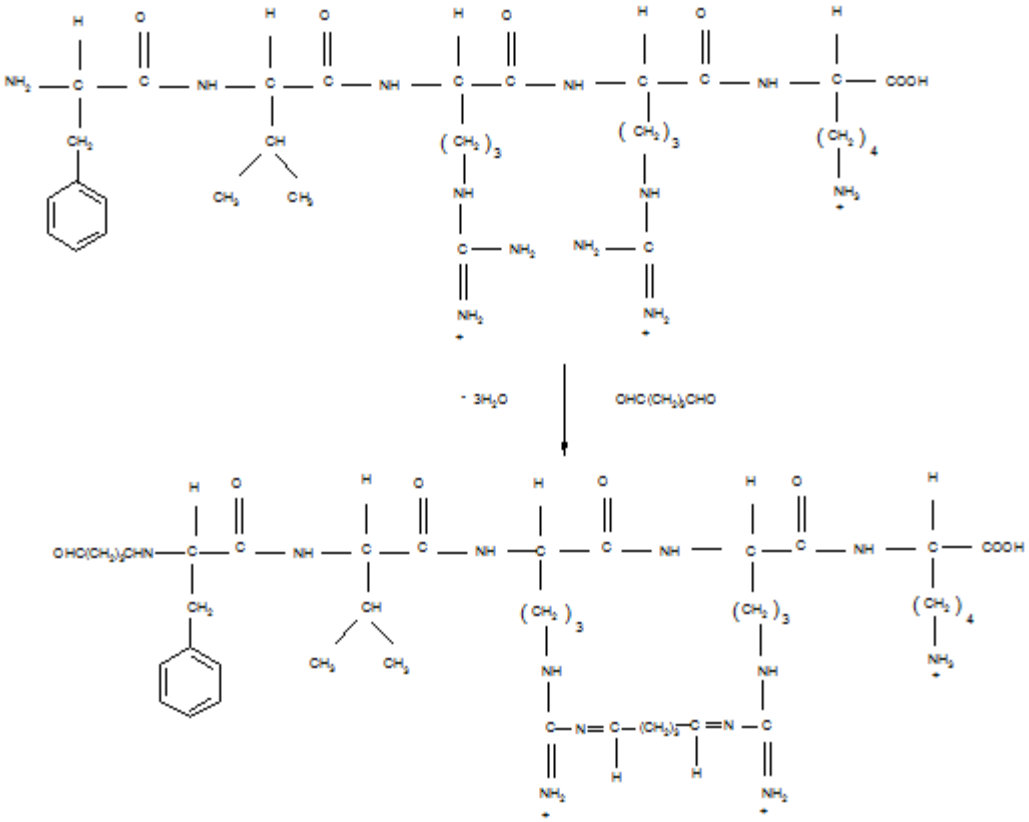

**Fig. 4: Effect of glutaraldehyde on water absorption of (a) soy protein isolate and (b) soy protein concentrate films plasticized by glycerol.**

80 %). Changes in the dimension of solution casted soy protein films due to swelling were also observed. On the other hand, swelling in compression molded films was not observed. Addition of different amount (0.4 to 2 %) of glutaraldehyde to GSPC-25 (S)/ GSPC-25 (C) or GSPI-25 (S)/ GSPI-25 (C), increased the water resistance property of soy protein films significantly. Water uptake of GSPC-25 was lower than that of GSPI-25. This may be due to higher amount of cellulose present in SPC. Cellulose is crystalline in nature and it absorbs less moisture in comparison to protein.

The mechanism of reaction between soy protein and glutaraldehyde is given in scheme 1. Soy protein contains 18 different amino acids out of 20 amino acids present in natural proteins. Amino acids such as asparagine, glutamine, lysine and arginine contain more than one $-NH_2$ group which facilitates the reaction with glutaraldehyde. To show the crosslinking reaction, a small unit of amino acid sequences (KVRRF represented lysine, valine, arginine, arginine and phenylalanine respectively) of $\alpha/\beta$ subunits of 11S globulin is taken and reacted with glutaraldehyde to give crosslinked soy protein.

**Table 4: Thermal characterisation of native and plasticized soy protein.**

| Sample | Mass loss (%) in the temperature range of | | | | Yc (%) |
|---|---|---|---|---|---|
| | 50-150°C | 150-200 °C | 200-300 °C | 300-400 °C | at 600°C |
| SPI | 7.3 | 1 | 16.7 | 27.7 | 30.3 |
| SPC | 5 2 | 2 | 12.3 | 37.8 | 25.1 |
| GSPI-50 | 11.8 | 4.1 | 23.8 | 32.0 | 17.4 |
| GSPC-50 | 12.2 | 8.8 | 26.7 | 23.0 | 16.2 |



**Scheme 1: Crosslinking reaction of soy protein with glutaraldehyde.**

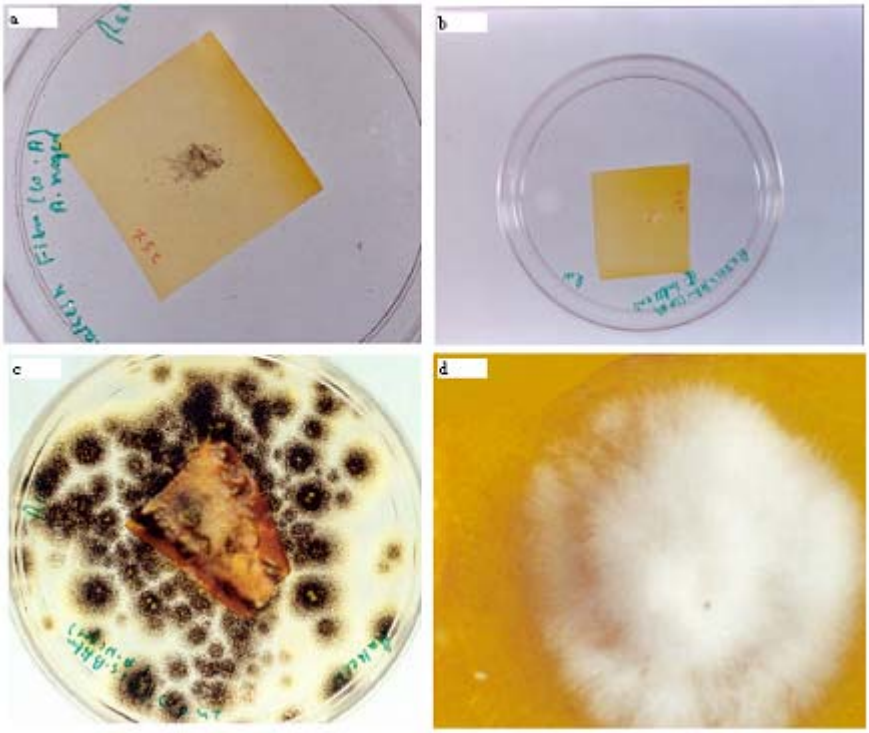



**Fig. 5: No growth of (a) *Aspergillus niger***
**(b) *Cyathus bulleri* on GSPC-25 film even after 6 months. Growth of**
**(c) *Aspergillus niger* (d) *Cyathus bulleri* on GSPC-25 film mounted on PDA media after 48 h.**

**Microbial Growth**

Figure 5 shows the microbial growth on soy protein films. No growth of microorganisms was observed even after 6 months, when autoclaved GSPC-25 film was used as the nutrients for growth of *A. niger* and *C. bulleri* (Figure 5a, b). This showed excellent stability of the GSPC-25 film against fungal growth. However, when autoclaved GSPC-25 films were mounted on solid PDA media, growth of *A. niger* and *C. bulleri* was observed on GSPC-25 film after 48 h. Figure 5c, d shows the growth of *A. niger* and *C. bulleri* on GSPC-25 films mounted on PDA media. Maximum growth was observed after 72 h of culturing. Thus, we can conclude that soy protein films will be not attacked by microrganisms when we use it judiciously. On the contrary, when we throw it in the environment contaminated by microrganisms, it can degrade very quickly.

**Conclusions**

Among all the polyols used, trimethylol propane and polyethylene glycol of higher molecular weight showed selectivity towards the soy protein for the formation of the films by solution casting method. Glycerol has been found to be better performing plasticizer as it forms film with both soy protein concentrate or soy protein isolate. Slight improvement in the mechanical properties, were observed for enzyme modified films except for papain modified film. Thermal stability of samples prepared by either solution casting or compression molding almost remained same. Hence, low cost solution casting method can be used to prepare soy protein films.

**ACKNOWLEDGMENTS**

ICI India Research and Technology Centre is gratefully acknowledged for sponsoring this project and providing financial assistance to one of the authors (Rakesh Kumar)

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