

*Enzymatically Transformed Soy Protein Binders and Banana
Fibre Reinforced Composites*

by
Rakesh Kumar

Centre for Polymer Science and Engineering

Submitted
In fulfillment of the requirements for the award of the degree of
Doctor of Philosophy
To the



Indian Institute of Technology, Delhi
October 2005

protein - Food stuffs
are reinforced & nutrients

I. I. T. DELHI.
LIBRARY
Acc. No. TH-3312

I.I.T. DELHI
LIBRARY
PROCESSED

TH
664.38
RAK-E

Dedicated to.....

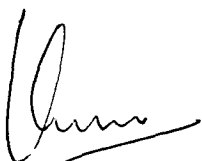
My Family



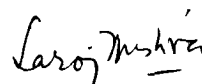
CERTIFICATE

This is to certify that the thesis entitled “*Enzymatically Transformed Soy Protein Binders and Banana Fibre Reinforced Composites*” being submitted by Mr. Rakesh Kumar to the **Indian Institute of Technology, Delhi**, for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him. Mr. Rakesh Kumar has worked under our supervision and fulfilled all the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The work embodied in this thesis has not been submitted, in part or full to any other University or Institute for the award of any degree or diploma.



Prof. (Mrs) Veena Choudhary
Centre for Polymer Science and Engg.



Prof. (Mrs) Saroj Mishra
Department of Biochemical Engg.
and Biotechnology

Indian Institute of Technology, Delhi

INDIA

ACKNOWLEDGEMENT

I acknowledge with a deep sense of gratitude to my thesis supervisors Prof. (Mrs.) Veena Choudhary and Prof. (Mrs.) Saroj Mishra for their constant inspiration, invaluable suggestions and motivating guidance for carrying out this work. Their cooperation and timely guidance were instrumental in presenting this work in the form of thesis. I shall be highly grateful to them.

My sincere thanks to Prof. (Mrs.) I.K. Varma for her valuable advices whenever required during the period of my research. I am indebted to Prof. A.K. Gupta, Prof. S.N. Maiti and Prof. A.K. Ghosh for their concern of my work.

I wish to thank Dr Vishal Anand, Dr Anjali Solanki, Dr Sandeep Tyagi, Dr Mona Malik, Dr Preeti Jain, Bimlesh Lochab, Dr Bharti Gaur, Nimisha Agarwal, Rashmi Chauhan, Joy Kishore Paul, D. Poornima, Smita Sangam, Kavita Abrol, G.S. Anantha, Senthil Kumar.

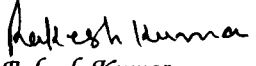
I am grateful to the staff of CPSE Mr. Surendra Kumar, Mr. Shiv Kant, Mr. Devendra Singh, Mr. A Sethi and Mr. Ashok Kapoor for their help during the work. I also thank Mr. Prabhu Nath Prasad for tracing the figures.

I am thankful to Dr Rajeev Rana, Dr Sanjeev Sharma, Dr Mukesh Kumar, Rajeev Kumar, Kranti Chawla, Dr Anubhav Saxena, Nihar Ranjan Kundu, Pabak Nanda, Anoop Kulsheshttra for their constant moral and technical support and keeping me cheerful during the hard days.

I am grateful to all my family members, especially my wife (Varsha Singh) and daughter (Yukta Singh) for their constant motivation.

Last, but not the least, I am sincerely grateful to my beloved parents for their constant moral support throughout my academic career.

I would like to acknowledge ICI R & T Centre, India for providing me financial support throughout the period.


Rakesh Kumar

ABSTRACT

Soybean is primarily an industrial crop cultivated for oil and protein production. The proportion of soy protein consumed directly in human nutrition and in other industrial uses is really small and there is a need to look for new industrial uses of soy protein. Plywood industry needs environment-friendly adhesives from renewable resources because petroleum resources are finite and are becoming limited, whereas the demand for adhesives is increasing. On the other hand, abundant proteins are available from renewable resources and agricultural processing by-products such as soy proteins from oil processing. Utilisation of these protein by-products as biodegradable adhesives and resins will help to overcome environmental problems and add value to agricultural by-products.

The present work was undertaken to look into the feasibility of using the soy protein as adhesives and evaluation of its performance as matrix resins in banana fibre reinforced composites. The effect of temperature, pH, time of mixing and centrifugation cycle on the recovery of soy protein isolate (SPI) from soy protein concentrate (SPC) was investigated in detail. The optimum pH and temperature for the recovery of protein were found to be 11 and 55°C respectively. Increase in these parameters resulted in a decrease in the protein recovery. Enzymatic modification of soy protein was carried out by treatment with different enzymes (papain, chymotrypsin, trypsin, urease and pepsin). Structural and molecular characterisation was done by SDS-PAGE, ion-exchange chromatography, hydrophobicity index and infrared spectroscopy. Chymotrypsin modified soy protein isolate (CSPI) showed only one molecular mass band of 24 ± 1 kDa which was of low intensity and represented the basic subunit of 11S-RG fraction. This suggested extensive

hydrolysis of SPI by chymotrypsin. High molecular mass bands of 63.4-79.4 kDa and 85-89 kDa were absent in trypsin modified SPI (TSPI). Papain modified SPI (PSPI) showed extensive hydrolysis after 1 h duration and all the significant molecular mass bands were absent with the exception of 23-25 kDa band. The 11S-RG had higher proportion of positively charged proteins than the 7S-RG fraction. At higher ionic strength, concentration of recovered protein was higher in the case of SPI followed by the 7S-RG and the 11S-RG fractions indicating the presence of more negative amino acids. The hydrophobicity index determined by the SDS binding method was found to be highest for CSPI and lowest for SPI. Hence, the number of exposed hydrophobic groups was concluded to be much more in the case of CSPI than in case of PSPI and TSPI.

In the FTIR spectra, ratio of the intensity of $>C=O$ of carboxyl group (1733 cm^{-1}) and amide I band (1652 cm^{-1}) / or amide II (1558 cm^{-1}) band was used to investigate the degree of hydrolysis. The ratio was found to be maximum in pepsin modified SPI (PeSPI) followed by chymotrypsin modified SPI (CSPI) suggesting that the extent of hydrolysis was highest in the case of pepsin modified SPI.

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were used for thermal characterisation of the soy protein and enzyme modified soy protein. In the DSC scans (recorded at a heating rate of $10^{\circ}\text{C}/\text{min}$) of the soy protein and its various enzyme hydrolysed products, one broad endothermic transition was observed below 120°C . SPI, SPC, 7S-RG and 11S-RG fractions showed broad single endothermic transition in the temperature range of $35\pm 5^{\circ}\text{C}$ to $135\pm 10^{\circ}\text{C}$. The enzymatic hydrolysis of soy protein i.e. samples CSPI or USPI had peak endothermic temperatures (T_p-1) higher than the soy protein.

The thermal stability of various samples was studied and multi-step mass loss behaviour was observed in all the samples. In all the soy protein samples, a mass loss of $7\pm 1\%$ was observed below 150°C with T_{max} values around $82\pm 10^{\circ}\text{C}$ except in CSPI where an exceptionally high value of 168°C was observed. A second endothermic transition in the temperature range of $197\text{-}223^{\circ}\text{C}$ was also observed in all enzymatically hydrolysed samples. This transition was associated with the degradation of polypeptide chains as indicated by accompanied mass loss in TGA. A sharp exotherm was observed above 200°C in all the samples.

The Brookfield viscosities of the prepared SPI adhesives were determined by Brookfield viscometer (Model DV-2). All the three liquid adhesive samples (I-12, I-13, I-14) and the commercially available adhesive showed shear thinning behavior. A decrease in the Brookfield viscosity was observed with increasing storage time of the SPI adhesive sample. Brookfield viscosity of enzyme modified SPI solution was lower as compared to SPI solution; however all the enzyme modified adhesive solutions showed almost similar value of viscosity except CSPI, suggesting that specificity of enzymes for hydrolysis did not affect the viscosity behaviour of the samples.

Adhesive strength of the soy protein and modified soy proteins on different types of wood substrates i.e. rubberwood, Bhutanpine, teakwood and plywood, was measured. A significant increase in shear strength (from 6.2 ± 2 to 14.68 ± 2 MPa) was observed on increasing the pH of the I-14 adhesive from 8 to 10. I-14 adhesives showed the best shear strength with rubberwood (14.68 ± 2 MPa) and poor with teakwood (4.86 ± 2 MPa). Incorporation of CMC and plasticizers in I-14 adhesive resulted in an increase in the adhesive strength.

Plasticized soy protein film and banana fibre reinforced soy protein composites were fabricated using plasticized soy protein as matrix resins. SPI and SPC film in the absence as well as in the presence of varying amounts of plasticizers (glycerol, PEG, TMP) were prepared. Films having thickness of 0.24 ± 0.05 mm were prepared by solution casting or compression molding. Brittle films were obtained in the absence of plasticizers whereas upon incorporation of plasticizers, an increase in % elongation after a subsequent decrease in tensile strength was observed. Enzymatic hydrolysis resulted in an increase in tensile strength except in the case of PGSPC-25 (S) i.e. papain modified SPC having 25 % (w/w) glycerol as plasticizer. Film could not be obtained in case of CGSPC-25 (S).

Thermal characterization of plasticized soy protein was done using DSC and TG. In the DSC scans of soy protein and its various plasticized products, one broad endothermic transition was observed below 120°C . SPI showed two broad endotherms with peak temperatures i.e. T_{p-1} and T_{p-2} at 60.1 and 220°C whereas SPC showed only one broad endotherm with peak temperature of 68.0°C . Incorporation of different plasticizers in SPI powder showed significant change in T_{p-1} and T_{p-2} which may be attributed to hydrogen bonding between soy protein and polyols. Plasticized films prepared by solution casting showed two endothermic transitions, except GSPI, in which a third endothermic transition was observed at 235°C . Thermogravimetric analysis showed multi-step mass loss behaviour was observed in all the samples. Plasticized samples displayed higher mass loss from room temperature to 150°C in comparison to the unplasticized samples.

Banana fibres (BF) were modified by treatment with sodium hydroxide. Morphological characterization (SEM) of the banana fibre presented a clean and smooth surface whereas unevenness of surface was observed after alkali treatment. The structural characterization

of fibres before and after treatment was done using FTIR and X-ray. An increase in tensile strength (~8 %) and modulus (~21 %) of the fibre was recorded upon treatment with 10 % sodium hydroxide. Percentage elongation of the fibre decreased indicating more brittle nature of the treated fibre. A single step degradation was observed in the treated (S-10-BF) and untreated fibre (BF).

The tensile strength and modulus of banana fibre reinforced soy protein composites was highest with SPI matrix having 25 % (w/w) glycerol. Further increase in glycerol content resulted in a decrease in the tensile strength and modulus. As the length of the S-10-BF fibre increased from ~0.5 to 11 cm, tensile strength and modulus showed an increase with soy protein matrices having 25-50 % (w/w) glycerol.

Addition of glutaraldehyde or IPDI increased the water resistance property of the native soy protein film and soy protein composites significantly. No microbial growth was observed even after 6 months, when GSPC-25 or G50CBF-3.0 film either autoclaved or unautoclaved was used as the nutrients for the *A. niger* and *C. bulleri*. This showed excellent stability of GSPC-25 or G50CBF-3.0 film against fungal growth.

CONTENTS

CERTIFICATE		i
ACKNOWLEDGEMENT		ii
ABSTRACT		iii-vii
LIST OF FIGURES, TABLES and SCHEMES		viii-xii
CHAPTER - 1	Introduction and Literature Survey	1-47
	1.1 Introduction	1
	1.2 Properties of Soy Protein	3
	1.3 Soy Protein Products	5
	1.4 Performance of Soy Protein Adhesives	9
	1.5 Modification of Soy Proteins	16
	1.6 Blends of Soy Proteins	27
	1.7 Soy Protein Based Plastics	31
	1.8 Applications	36
	1.9 Objective of the Work	37
	1.10 Format of the Thesis	38
	References	39
CHAPTER – 2	Proteolytic Modification of Soy Protein and its Molecular Characterisation	48-67
	2.1 Introduction	48
	2.2 Experimental	49
	2.2.1 Materials	49
	2.2.2 Preparation of Soy Protein Isolate (SPI) from Soy Protein Concentrate (SPC)	50

2.2.3	<i>Preparation of 7S-RG and 11S-RG Fractions</i>	51
2.2.4	<i>Extraction of Soy Protein from Black Soybean Seeds</i>	51
2.2.5	<i>Enzymatic Modification of Soy Protein Isolate</i>	51
2.3	Characterisation Techniques	52
2.3.1	<i>Protein Content</i>	52
2.3.2	<i>Solid Content</i>	52
2.3.3	<i>Clarity</i>	53
2.3.4	<i>Molecular Mass Determination</i>	53
2.4	Results and Discussion	54
2.4.1	<i>Protein Content</i>	54
2.4.2	<i>Clarity</i>	59
2.4.3	<i>Molecular Characterisation</i>	60
	References	67
CHAPTER – 3	Structural and Thermal Characterisation of Modified Soy Protein Isolate	68-84
3.1	Introduction	68
3.2	Experimental	69
3.2.1	<i>Structural Characterisation</i>	69
3.2.2	<i>Thermal Characterisation</i>	71
3.3	Results and Discussion	71
3.3.1	<i>Structural Characterisation</i>	71

	3.3.2 <i>Thermal Characterisation</i>	76
	References	84
CHAPTER - 4	Evaluation of Native and Modified Soy Protein Isolate as Wood Adhesives	85-99
	4.1 Introduction	85
	4.2 Experimental	86
	4.2.1 <i>Materials</i>	86
	4.2.2 <i>Preparation of SPC Adhesive</i>	86
	4.2.3 <i>Preparation of Soy Protein Isolate Adhesive</i>	87
	4.2.4 <i>Preparation of Soy Protein Isolate Adhesive Modified by Plasticizers and Thickeners</i>	87
	4.3 Characterisation Techniques	88
	4.3.1 <i>Viscosity</i>	88
	4.3.2 <i>Shear Strength</i>	88
	4.4 Results and Discussion	89
	4.4.1 <i>Viscosity</i>	89
	4.4.2 <i>Shear Strength</i>	93
	References	99
CHAPTER - 5	Plasticized Soy Protein Film and Banana Fibre Reinforced Soy Protein Composites: Fabrication and Characterisation	100-135
	5.1 Introduction	100
	5.2 Experimental	101
	5.2.1 <i>Materials</i>	101

5.2.2	<i>Separation and Modification of Fibres</i>	102
5.2.3	<i>Preparation of Soy Protein Films</i>	102
5.2.4	<i>Enzymatic Modification of Soy Protein Concentrate</i>	104
5.2.5	<i>Preparation of Composites</i>	104
5.2.6	<i>Water Absorption</i>	105
5.2.7	<i>Microbial Study</i>	106
5.3	Characterisation of Fibres	106
5.4	Characterisation of Film and Composites	108
5.5	Results and Discussion	109
5.5.1	<i>Characterisation of Banana Fibre</i>	109
5.5.2	<i>Characterisation of Soy Protein Films and Composites</i>	115
	References	134
CHAPTER - 6	Summary and Conclusions	136-146
CIRRICULUM VITAE		147-148