BIODEGRADABLE POLYMERS
Part VI. Biodegradable plastics of soy protein isolate modified with thiourea

P. K. Nanda[1], K. K. Rao[2], R. K. Kar[1] and P. L. Nayak[1*]

[1]Biodegradable Polymer Research Laboratory, P.G. Department of Chemistry, Ravenshaw College, Cuttack 753 003, India
[2]Regional Research Laboratory (CSIR), Bhubaneswar, India

Potential alternative to petrochemical polymers, soy protein isolate (SPI), a plentily available, natural biopolymer is chemically modified with thiourea at 2.5, 5, 7.5, 10, 15 and 20 mass/mass% for better processing of plastic as a raw material. From the FTIR studies, it has been ascertained that there is no bonding reaction between SPI and thiourea and it acts as a modifier only. Thermogravimetric analysis of the modified material has been followed using a computer analysis method, LOTUS package, developed by us for assigning the degradation mechanism. A number of equations have been used to evaluate the kinetic parameters. The mechanism of degradation of the biopolymer is explained on the basis of the kinetic analysis.

Keywords: *biodegradation, soy protein isolate, thermogravimetric analysis, thiourea*

Introduction

Development of biodegradable polymers from renewable resources to replace conventional synthetic plastic products provide opportunities for reducing waste through biological recycling to the biosystem. Use of biodegradable polymers is also seen as an approach for slowing the introducing of fossil-fuel-derived carbon dioxide into the atmosphere. Biological recycling of biodegradable polymers is an important option for reducing municipal solid waste. Biodegradable polymers based on cellulose, starch, protein, microbial polyesters and polylactic acid each have novel properties, which suggest possible applications.

Abundant plant-based proteins are available from renewable resources and agriculture processing by-products, such as soybean proteins from oil processing and gluten proteins from corn or wheat-starch production. For example, soybean contains about 40% protein, and the United States produces about 52% of the total world soybean crops. Utilizing these protein by-products for the manufacture of biodegradable resins will help alleviate the environmental problems and add value to agricultural by-products [1–5].

Soy proteins have commonly been used for food and animal feed for many years. However, soy protein is a new biopolymer for synthesizing biodegradable resins. Soy protein polymers are macromolecules that contain a number of aminoacids at the side chains. Major protein components include 2S, 7S, 11S and 15S fractions, classified by their ultra-centrifugal sedimentation rates.

The 7S fraction makes up approximately one third of the total soybean proteins, and its main component is 7S globulin. The 11S fraction is roughly 50% of soybean proteins, and contains a single component called 11S globulin [6]. Physiochemical properties of 7S and 11S globulin have been extensively studied in food applications [7–13].

Soy protein possesses many side reactive groups such as $-NH_2$, –OH and –SH which are 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 were 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 [17]. Nitrogen compounds like urea is often used as denaturing agent for protein.

* Author for correspondence: nayakpl@sify.com

1388–6150/$20.00
© 2007 Akadémiai Kiadó, Budapest
Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

A survey of relevant literature reveals that thiourea has never been used as denaturing agent for the modification of proteins. Thiourea possesses many better properties than urea because of the presence of sulphur atom in the molecule. It can destabilize globular protein by forming strong hydrogen bonds with water molecules that surround the protein and may protect it from denaturing while disrupturing protein hydrogen bonds, resulting in partially unfold protein structures and flexible peptide bonds [18]. Some nitrogen and sulfur compounds like urea and thiourea could be used as modifiers for better processing conditions and commercial applications.

In the present research programme, thiourea has been used as a modifier to modify soy protein isolate for better commercial values. The degradation behaviour of the modified soy protein isolate has been monitored by TG analysis. A novel LOTUS package computerized method developed by us [3–5] 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.

Experimental

Materials

SPI with protein content of about 90% was obtained from Archer Daniels, Midland Co., [Decatur, IL, USA] as a gift sample and used for the reaction. Thiourea (GR), obtained from Germany (Merck), was used for modifying the protein molecule.

Preparation of modified soy protein isolate

Soy protein isolate powder (at a mass ratio of 1:10 of deionised water) was added to solutions of thiourea [(00, 2.5, 5, 7.5, 10, 15 and 20 mass/mass%) dry base of SPI] while stirring. The slurry was continuously stirred with a mechanical stirrer for 6 h at room temperature and was allowed to stand for 18 to 24 h. Then the pH of the slurry was adjusted to 4.5 by adding propionic acid dropwise, while the mixture was continuously stirred. Since it is the isoelectric point of the protein, at this pH value, it has the least net charges and is most water resistant. The slurry was then centrifuged to remove excess water (Sorvall Superspeed RC2-B; 4541 g, 10 min) and the precipitated residue was dried for 24 h in a convection oven at ≤50°C. The dried modified soy protein isolate was then milled (Cyclone Sample Mill, UDY Corporation Fort Collins, Co.) to pass through a 35 mesh sieve.

Methods

Infrared spectra

Fourier-transform infrared (FTIR) spectra were recorded with Perkin-Elmer 1720 spectrophotometer using KBr pellets.

Thermogravimetric analysis

Thermal degradation pattern of the modified biopolymers were studied using thermogravimetric analyser (TGA7, Perkin Elmer, Norwalk, CT) in N_2 atmosphere. The temperature range for scanning was from room temperature to 800°C at 10°C min^{-1} increment. In the present investigation, a program developed by us using MACROS, has been used to calculate the kinetic parameters from the non-isothermal TG curves. After feeding the values of corresponding temperatures and number of data points, the program calculates $\ln g(\alpha)/T^2$, $1/T$, slope, intercept, R^2 and error values for all the thirty reaction mechanisms [4] and prepares corresponding charts.

Results and discussion

The thermal degradation data of the modified soy protein are furnished below in Table 1.

FTIR of the resins

FTIR spectra of the SPI resins, FTIR-01 and FTIR-07 (Figs 1a and b) containing thiourea 0 and 20% respec-

Table 1 Thermal decomposition data of modified SPI with thiourea in propionic acid medium

Sample code	Thiourea/%	Mass loss% at various temperatures/°C							
		100	200	300	400	500	600	700	800
PKN-01	0.0	8	12	25	55	63	68	76	83
PKN-02	2.5	6	11	22	60	71	73	75	76
PKN-03	5.0	5	10	21	62	72	74	75	77
PKN-04	7.5	9	11	24	61	71	74	75	77
PKN-05	10	3	7	20	53	62	66	70	75
PKN-06	15	8	11	25	61	70	72	74	75
PKN-07	20	8	11	25	53	62	69	78	82

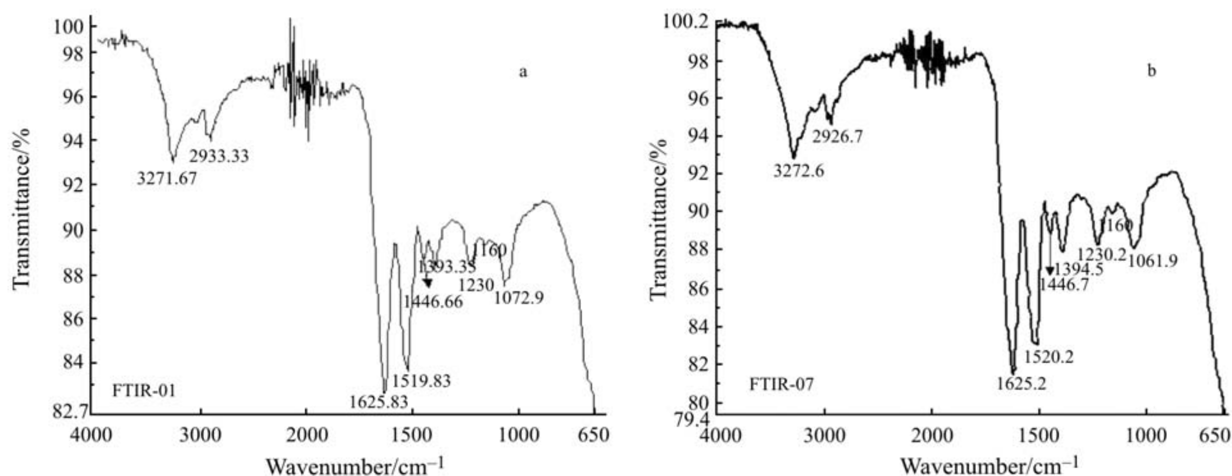

Fig. 1 FTIR spectra of SPI modified with thiourea a – 0 and b – 20%

tively, show an absorption band at 3271 cm^{-1}. This is due to the N–H stretching of the amine [22]. The band at 1393 cm^{-1} is due to C–N stretching of the free amines present in the resin. The band around 1060 cm^{-1} in FTIR-07 is due to the C=S band of the thiourea present in the compounding mixture. It is well known that, proteins of mainly alpha helical structures may exhibit an absorption band around 1650 cm^{-1}. Protein structure is also considered as an unordered random coil with absorption from 1655 to 1658 cm^{-1}. There is no such band with the thiourea-modified resin. Parallel beta pleated sheets in polypeptide chains are characterized by binding with absorption around 1625 cm^{-1}. In case of FTIR-07, scan exhibited a peak at 1625 cm^{-1} indicating that a dispersion of unordered random coils, helical coils and beta pleated sheets forming an amorphous structure.

TG analysis

The kinetic parameters such as '*A*', '*E*' and 'frequency factors' are calculated for all the modified products and the data are presented in Table 2.

The data on temperature and percent mass loss have been subjected to differentiation to fix the actual number of stages involved in the process. Percentage mass loss was differentiated with respect to temperature and each time change in the sign of slope observed, it is presumed that there is a change in chemical reaction.

Model considered

The data has been analyzed to study the best fit model among the 30 models [4]. For fixing the best fit model, linear regression analysis has been carried out on all the models; the model that has R^2 closest to one has been chosen as the best fit model. When R^2 is identical for any of the two or more models, error in estimation of slope and constant were taken into consideration and the model that has least error in estimation of slope and constant, has been chosen to be the best fit.

The degradation of the unmodified and thiourea modified soy protein isolate could be dissected into four steps (Figs 2a–g). For example, as in case of sample PKN-03, the first thermal degradation takes place around 105°C having mass loss about 6%, the second break takes place around 295°C having mass loss of about 20%, the third break takes place around 377°C having mass loss about 54% and the fourth break takes place around 800°C having mass loss around 80%. All other samples exhibit the same degradation mechanism. 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 are 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].

The first break around 105°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 106 to 295°C is mainly due to the

Table 2 Kinetic parameters of the modified SPI with thiourea

Sample no.	Temperature range/°C	Model	Slope	Intercept	R^2	E/kJ mol^{-1}	Frequency factor
PKN-01_1	38–105	B1	–1097.7	8.2249	0.9863	20.10	12508.0
PKN-01_2	106–250	B1	–849.4	7.1167	0.9509	15.56	3195.5
PKN-01_3	251–353	B1	–2325.5	9.4523	0.9708	42.59	90425.3
PKN-01_4	354–800	B1	–1244.6	7.3215	0.9740	22.80	5746.4
PKN-02_1	36–105	H4	–1392.9	8.7745	0.9992	25.51	27499.4
PKN-02_2	106–278	P1	–738.9	6.8095	0.9544	13.53	2044.4
PKN-02_3	279–374	B1	–2471.4	9.5743	0.9701	45.27	108559.9
PKN-02_4	375–800	B1	–1204.4	7.3452	0.9618	22.06	5694.2
PKN-03_1	36–105	B1	–1036.8	8.0292	0.9959	18.99	9713.6
PKN-03_2	106–295	P1	–755.5	6.8199	0.9617	13.84	2112.2
PKN-03_3	296–377	B1	–2793.0	10.0553	0.9642	51.16	198479.3
PKN-03_4	378–800	B1	–1184.4	7.3257	0.9648	21.69	5491.5
PKN-04_1	37–114	H4	–1092.7	7.9486	0.9992	20.01	9444.7
PKN-04_2	115–226	B1	–989.8	7.4542	0.9546	18.13	5218.8
PKN-04_3	227–380	B1	–1764.9	8.4564	0.9782	32.33	25349.5
PKN-04_4	381–800	B1	–1231.7	7.3685	0.9645	22.56	5960.2
PKN-05_1	42–127	B1	–1382.8	8.8671	0.9269	25.33	29947.5
PKN-05_2	128–213	B1	–1153.7	7.8416	0.9347	21.13	8960.1
PKN-05_3	214–362	B1	–1691.7	8.3856	0.9879	30.98	22635.8
PKN-05_4	363–802	B1	–1237.9	7.3342	0.9714	22.67	5788.4
PKN-06_1	38–105	B1	–1029.9	8.0359	0.9915	18.86	9714.4
PKN-06_2	106–257	P1	–755.0	6.8575	0.9501	13.83	2191.8
PKN-06_3	258–366	B1	–2238.6	9.2550	0.9712	41.00	71458.2
PKN-06_4	367–803	B1	–1200.8	7.3368	0.9673	21.99	5629.5
PKN-07_1	42–119	B1	–1052.7	8.0403	0.9835	19.28	9972.9
PKN-07_2	121–221	B1	–979.4	7.4462	0.9437	17.94	5122.4
PKN-07_3	223–355	B1	–1857.7	8.6852	0.9768	34.02	33540.9
PKN-07_4	357–747	B1	–1315.8	7.4129	0.9703	24.10	6656.5

cleavage of the covalent bonding between the peptide bonds of amino acid residues. During this period 60% of phenyl-alanine 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 296–377°C is probably due to cleavage of S–S, O–N and O–O linkages of the protein molecule. The fourth break between 378–800°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.

It is observed in sample PKN-03 that, the initial, third and fourth steps followed B1 (Prout–Tompkin law) mechanism while the second step follows P1 (power law) 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 curve substantiated by the mechanism takes place in four steps. The values of the activation energy in the first step is comparatively high indicating slow degradation process in case of all the seven samples. 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, electrostatic and other weak bonds most probably takes place quicker 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 thereby decreasing the rate of the reaction. Hence the probable thermal degradation pattern of the modified soy protein isolate agrees well with the predicted mechanism as evident from the computerised values of the energy of activation.

In case of unmodified SPI, the first thermal degradation takes place at 105°C having mass loss of about 8%. The second thermal degradation step occurs at 250°C with mass loss of about 20%. The third break takes place at 353°C with 40% mass loss and fourth break takes place at 800°C with mass loss of 83%. The degradation pattern is the same as with modified SPI.

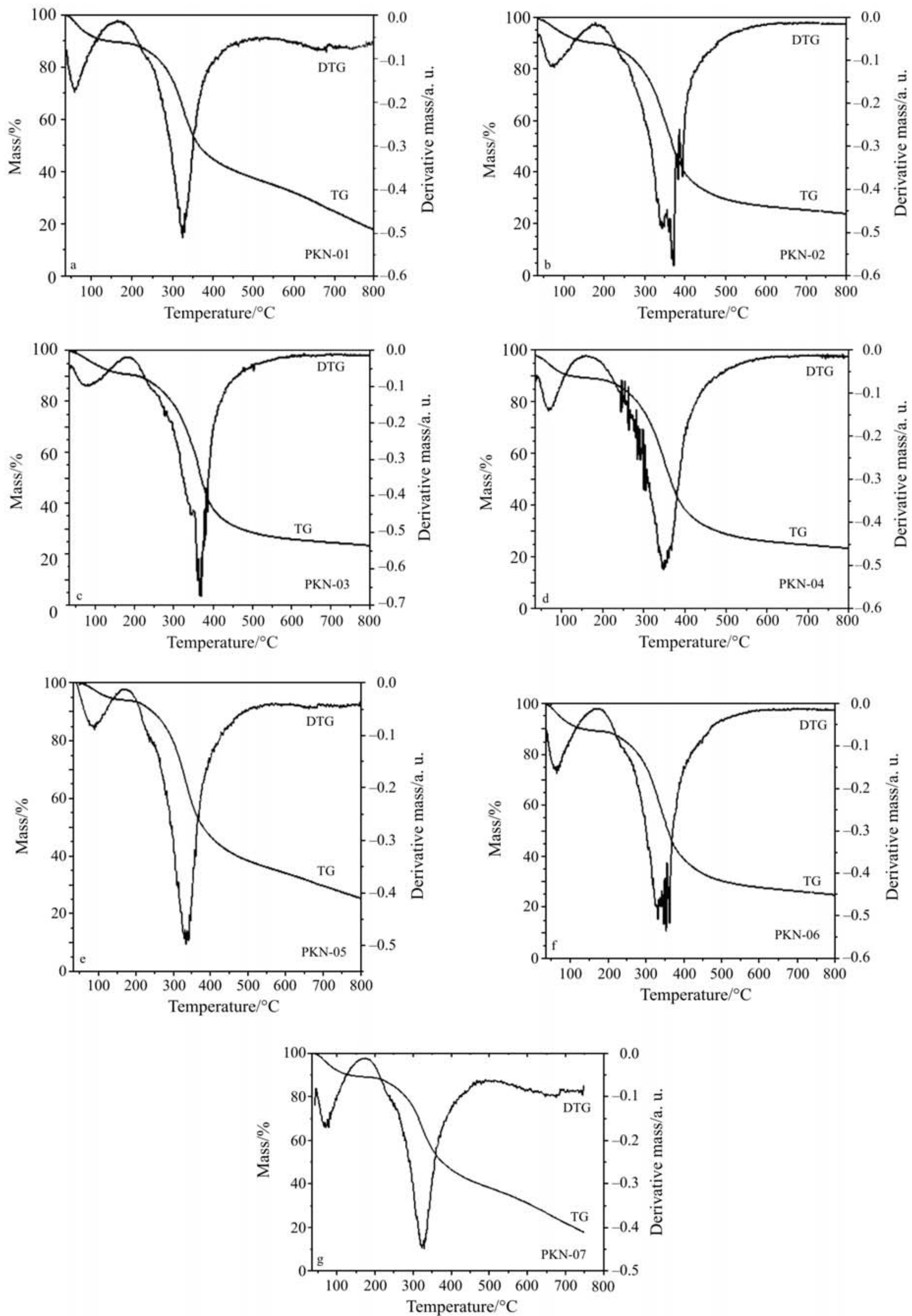

Fig. 2 TG and DTG curves of SPI modified with thiourea a – 0, b – 2.5, c – 5, d – 7.5, e – 10, f – 15 and g – 20%

The thermal degradation pattern of unmodified SPI follows the B1 (Prout–Tompkin law) mechanism in all the four steps and the kinetic parameters follow the same pattern as that of modified SPI. For example, the values of energy of activation decreases in the second step, then increases and decreases in the fourth step, which is similar to PKN-03.

Conclusions

SPI has been modified using a sulfur containing compound such as thiourea at different mass%. From the FTIR studies, it is ascertained that thiourea does not show any chemical reaction with SPI, rather it acts as a modifier. The thermogravimetric analysis of the unmodified and modified SPI has been monitored with a view to ascertain the degradation pattern of the resin. A computerized LOTUS package method has been used to calculate the values of energy of activation of each step of degradation. The curves of the unmodified and modified SPI has been dissected into four stages and the mass loss in each step has been discussed considering the breakage of the groups present in them. Based on the values of energy of activation, the kinetic mechanism of each step of degradation has been depicted.

Acknowledgements

The authors are grateful to University Grants Commission, New Delhi, India, for financial assistance under Research Project No. F. PSO-024/03-04 (ERO).

References

1 P. L. Nayak, J. Macromol. Sci- Rev. Macromol. Chem. Phys., C39 (1999) 481.
2 S. N. Swain , S. M. Biswal, P. K. Nanda and P. L. Nayak, J. Polym. Environ., 12 (2004) 35.
3 S. N. Swain, K. K. Rao and P. L. Nayak, J. Appl. Polym. Sci., 93 (2004) 2590.
4 S. N. Swain, K. K. Rao and P. L. Nayak, J. Therm. Anal. Cal., 79 (2004) 33.
5 S. N. Swain, K. K. Rao and P. L. Nayak, Polymer International, 54 (2005) 739.
6 W. J Wolf , J. Agric. Food Chem., 18 (1970) 967.
7 R. C. Roberts and D. R.Briggs, Cereal Chem., 42 (1965) 72.
8 K. Saio, I. Satoh and T. Watanabe, J. Food Sci., 39 (1973) 777.
9 K. Saio and W. Watanabe, J. Texture Stud., 9 (1978) 135.
10 J. B.German, T. E. O'Neil and J. E. Kinsella, J. Am. Oil Chem. Soc., 62 (1985) 1358.
11 T. Bogracheva Ya, E. E. Braudo and V. B. Tolstoguzov, Food Hydrocolloids, 4 (1980) 1.
12 H. Zhang, M. Takenaka and S. Isobe, J. Therm. Anal. Cal., 75 (2004) 719.
13 R. Kumar, V. Choudhary, S. Mishra and I. K. Varma, J. Therm. Anal. Cal., 75 (2004) 727.
14 J. Bjorksten, Adv. Protein Chem., 6 (1951) 343.
15 S. Wang, H. J. Sue and J. L. Jane, Pure Appl. Chem., A33 (1996) 557.
16 S. Zhang, P. Mungara and J. Jane, Polym. Preprints, 39 (1998) 162.
17 Y. V. Wu and G. E. Inglett, J. Food Sci., 39 (1974) 218.
18 I. Hayakawa, Y. Linko and P. Linko, Lebensm. Wiss. Technol., 29 (1996) 756.
19 C. V. Morr, JAOCS, 67 (1990) 265.
20 A. Kilara and T. Y. Sharkasi, Crit. Rev. Food Sci. Nutr., 23 (1986) 323.
21 M. A. Moharram and K. N. Abd-El-Nour, Polym. Degrad. Stab., 45 (1994) 429.

Received: January 6, 2006
Accepted: April 7, 2006
OnlineFirst: August 11, 2006

DOI: 10.1007/s10973-006-7491-8