Annals of Plant Sciences
ISSN: 2287-688X
Original Article

Effect of pH on Physico-Chemical and Mechanical Properties of Composite Films

V Ramnath[1*], M Gajalakshmi[1], J lalitha[1], S Sekar[2] and S Sankar[2]
[1]PG & Research Department of Biochemistry, St.Josephs college of Arts and science, Manjakuppam, Cuddalore, Tamilnadu, India
[2]Bio-products Laboratory, Central Leather Research Institute, Adyar, Chennai-600 020, India.

Received for publication: May 05, 2013; **Accepted**: June 12, 2013.

Abstract: The effect of pH on physico-chemical and mechanical properties of composites films prepared from natural resources was reported in this study. Composite films varying in their pH (range 3-7) containing soya protein (SY) and sago starch (SG) were prepared by solvent casting method. These films were cross linked with glutaraldehyde to improve their mechanical properties. The films prepared were characterized for their water absorption, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), mechanical properties and thermogravimetric analysis (TGA). The results obtained showed that change in pH of the SG-SY-G composite from its native pH of 5, has significantly influenced the water absorption capacity, and rearrangement in the functional groups. An increase in the thermal stability was observed beyond the native pH and thermally unstable below the native pH (i.e pH3 and 4). The scanning electron microscope studies have shown a smooth surface of the SG-SY-G film in the pH range 3, 4 and 5 and presence of smooth surface with granules in the pH 6 and 7. This study revealed that change in the pH of soya protein from its native pH 5, has influenced notable changes in its physico-chemical and mechanical properties to that of other composites prepared in other pH range.

Keywords: pH; Composite film; soya protein; sago starch; Biomaterial.

Introduction

Naturally derived materials are becoming widely used in the biomedical field. Soya bean is one of the most important and widely consumed legume crops in the world. It is composed of approximately 38% proteins, 30% carbohydrates, 18% oil and 14% minerals, other materials and moisture [1]. Soy proteins, of vegetal origin, are composed of a mixture of albumins and globulins, 90% of which are storage proteins with globular structured consisting mainly in 7S (b-conglycinin) and 11S (glycinin) globulins. The 11S fraction consists of glycinin, the principal protein of soybeans. Glycinin has a molecular mass of 320–360 kDa. 11S is a quaternary structure composed of three acidic and three basic subunits with isoelectric points between pH 4.7–5.4 and 8.0–8.5, respectively. The polypeptides in native glycinin are tightly folded and stabilised via intermolecular disulphide bonds. The ability of soy proteins to undergo association–dissociation reactions under known conditions is related to their functional properties and particularly to their texturisation [2-5]. The solubility of soy proteins in water is strongly affected by the pH. About 80% of the protein in raw seeds or unheated meal can be extracted at neutral or alkaline conditions. The pH dependence of solubility is used in the manufacture of isolated soybean protein. As the acidity is increased, solubility drops rapidly and a minimum is observed at pH 4.2–4.6 which is the isoelectric region of soybean proteins [6].

Soy protein isolate (SPI) is widely tested as a film-forming material for its natural abundance as a co-product of the soybean oil industry [7].The use of SPI for film preparation could add value to soybeans. Although SPI can produce flexible, clear edible films with excellent oxygen and lipid barrier properties, films made from native soy proteins are rather brittle and have poor water vapour permeability [8]. The inadequate film-producing property is attributed to the presence of abundant free hydroxyl groups in the matrix, which interact strongly with migrating water molecules [9]. Soy protein can be cross-linked by a cross-linker, heat treatment, enzymatic treatment or irradiation. It has many reactive groups (e.g. –NH2, –OH and –SH) which enable cross-linking reactions. Low molecular

***Corresponding Author:**
Dr.V.Ramnath
Associate Professor,
PG & Research Department of Biochemistry,
St. Josephs college of Arts and Science,
Cuddalore.607001, Tamilnadu, India.

aldehydes (e.g. formaldehyde, glutaraldehyde and glyoxal) react primarily with the free Ɛ-amine groups of arginine, lysine and hydroxylysine residues of the protein, thereby forming intra- and intermolecular cross-links [10-13]. The addition of polyol plasticisers, which are often needed to alleviate the brittleness problem, tends to suppress the film formation due to their strong hydrogen bond interaction with proteins, hence, reduced protein–protein interaction in the film [14-15].

Among natural polymers, starch has been considered as one of the most promising candidates for the future primarily because of an attractive combination of availability, price and performance. Starch consists of the linear a-D-glucan amylose and highly branched amylopectin. Starches in their native forms, are organized into semicrystalline granules [16-18].The mechanical properties and water resistance nature of starch can be modified by blending with synthetic or natural polymers and by cross-linking [19-23]. Studies show that the preparation of composites can also improve the mechanical properties of starch [24-25]. Earlier reports indicate that the presence of a protein in a polysaccharide–protein blend may improve the cell adhesion response of the resultant material due to the presence of more protein-binding sites [26].

In our previous study we reported the physical and chemical interactions of composite film made of soya protein isolate in its native pH in combination with the polysaccharides sago starch cross-linked with glutaraldehyde. In the present investigation, soya protein filtrate differing in their pH was added to gluteraldehyde-plasticized sago starch and from this mixture plasticized soya/starch composite films were obtained. The goal of the present work, therefore, was to study the effect of change in pH of soya protein on physico–chemical and mechanical properties of composite films prepared.

Materials and Methods

Soya seeds and Sago rice were purchased from nearby local retail market and all the chemicals used in this study were of laboratory grade.

Preparation of soya solution:

The soya seeds were air dried and then powdered using a domestic mixer and sieved to a particle size of 50- 200µm**.** 2g of soy powder was dissolved in deionised water at room temperature and then boiled for 30mts.The temperature was maintained between 70-80^{o}c throughout the boiling process. The boiled soya solution was then cooled and filtered through a muslin cloth. The filtered solution was then adjusted to obtain the desired pH range of 3 and 4 with 2 M HCl, to pH 6 and 7 with 2 M NaOH [27]. The pH-adjusted protein solutions were kept for 1 h at room temperature to induce partial unfolding. The soya solutions varying in their pH was used for further experiments.

Preparation optimization and cross-linking of soya sago solution with gluteraldehyde (SG-SY-G)

Preparation, optimization and cross-linking of soya protein and sago starch with gluteraldehyde were reported in our earlier studies [28]. Briefly, 60 ml of 10%sago solution, 10ml of 2% soya solution, 2ml of ethylene glycol (EG) and 1µl of gluteraldehyde were mixed well and poured in polythene tray having measurements 10×10, and dried at 30-35^{0}C.

Preparation of SG-SY-G film at various pH ranges

The stoichiometric ratio of the composite film which gave better mechanical properties (Table 3, sample 2) [28] was used to prepare films varying in their pH. Here keeping the amount of sago solution, EG and glutaraldehyde constant the amount of soya solution varying in the pH was added and the details are given in the table 4.

Table.1: Sample composition and tensile properties of SG-SY-G film at various pH ranges

Sample No	Sago (ml)	pH of soya solution	Soya (ml)	EG (ml)	Gluteraldehyde (µl)	Elongation at break (%)	Tensile strength (MPa)
1	40	3	10	2	1	13.56	0.74
2	40	4	10	2	1	10.50	0.89
3	40	5	10	2	1	18.22	1.85
4	40	6	10	2	1	16.39	2.04
5	40	7	10	2	1	21.11	2.06

Characterization:

Water absorption capacity of the samples: Estimation of water absorption capacity was done by the method. The water absorption capacity of samples prepared was determined by swelling small pieces of each sample of known weight in distilled water at room temperature. The swollen weights of the samples were determined by first blotting the samples with filter paper followed by accurately weighing the sample. The weights of the swollen pieces were recorded every 1h, 2 h, and 3 h, and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula

$$\mathbf{ES} = \frac{\mathbf{Ws - Wo}}{\mathbf{Wo}} \mathbf{X\ 100}$$

Where Ws is the weight of the sample (moist) at given time, Wo is the initial weight of the sample, Es is the percent of swelling at a given time. The results given are average of three samples.

Infrared Spectroscopy:

FTIR spectra of the samples prepared were taken using Nicollet impact 400 FTIR spectroscopy by preparing a 500mg KBr pellet containing 2-6 mg of the sample.

Scanning electron microscopy (SEM):

The sample was coated with gold ions using an ion coater (fisons sputter coater) under the following conditions: 0.1 Torr pressure, 20 mA current and 70 s coating time. Surface structure was visualized by scanning electron microscope (JSM 5300 Scanning microscope) using a 15 KV accelerating voltage.

Tensile strength

Three dumbbell-shaped specimens of 4mm wide and 10mm length were punched out from the prepared films using a die. Mechanical properties such as tensile strength (MPa) and percentage of elongation at break (%) were measured using a universal testing machine (INSTRON model 1405). The results given are an average of three specimens [29].

Thermogravimetric analysis (TGA)

The thermal stability was determined with a thermo gravimetric (TG) analyzer (Perkin-Elmer TGA) over a temperature range of 37^0C to 800^0C at a heating rate of 20^0C / min under nitrogen atmosphere.

Total Soluble Matter

Film specimens from a film were directly immersed in water (25°C for 24 h) and subsequently oven dried (105°C for 24 h) to determine the solubilized dry matter. Initial dry matter values needed for TSM calculations were the ones obtained from MC measurements for the same film.

Film thickness

The thickness of the films was measured by using a screw gauge with 0.001 mm accuracy according to the method of Xu et al [30]. Five thickness measured were taken of each film one at the centre and four around the perimeter. Average values were used in the calculation.

Moisture Content

Film samples (0.2g) were weighed into aluminum crucibles and dried at 105^0C in an oven for 24 hours. Moisture content (MC) was determined as a percentage of the initial film weight lost during drying and was reported on a wet basis. Triplicate measurements of MC were obtained for each type of film.

Statistical Analysis

The statistical analysis data was expressed as the arithmetic mean ± standard deviation with n=6. The results were performed by using one-way analysis of variance (ANOVA). The significant difference level is $p \leq 0.05$.

Result and Discussion

The film-forming properties of SPI can be improved through ''pH-shifting'', i.e., exposing proteins to extreme acidic or alkaline pH, followed by neutralization, which could induce a molten globule protein conformation. Previous studies reported that incubation of SPI in a pH 1.5 or a pH 12 solution, then in a pH 7 solution for 1 h, produced structurally modified SPI that exhibited greater hydrophobicity as well as solubility, along with some soluble polymers formed [31-32].

Water Absorption Studies

Water absorption capacity of the samples composite film varying in their pH were tested for their water absorbing ability in time durations of 1, 2, 3 and 24 h. Fig 1 revealed that the composite film at pH 6 has shown increased water absorbing capacity of 144% with increase in time, after 24 h. Composite film prepared in other pH ranges

(3, 4, 5, and 7) also exhibited increased water absorption capacity (99, 93, 94, and 89% respectively). However, the water absorption capacity of composite film with pH 3, 4, 5 and 7 were less at all the times when compared to that of composite film of pH 6. This may be explained that decrease in water absorption capacity of the composite film with pH 3,4,5 and 7 may be due to unavailability of free hydroxyl groups on the polysaccharide backbone of sago starch. As the hydroxyl groups are more hydrophilic in nature increased water absorption capacity was observed in the pH range of 6 when compared to other pH ranges.

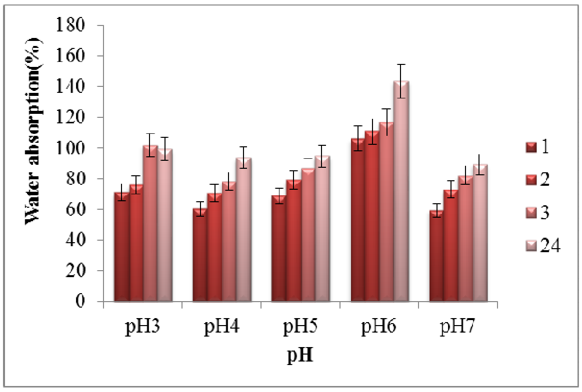

Fig.1: Water absorption studies of composite films. The data are presented as ± SD of 6 individual experiments

Tensile Strength:

Proteins are stabilised by hydrogen bonds, dipole–dipole, charge–charge, and hydrophobic interactions, and disulphide bonds in some cases [33]. These intramolecular forces cumulatively contribute to the conformational stability of soy proteins and are largely undisrupted with even moderate heating or change in pH [34]. The mechanical studies of various composite films (Table 2) which differ in their pH revealed that, the composite film with pH 5 exhibited better tensile property when compared to that of other films with pH 3, 4, 6 and 7. A comparative decrease in the tensile strength of the composites at all the pH range with respect to pH 5 revealed the stability of the soya protein at in its native pH (i.e. pH 5).The better tensile nature of the composite film at its native pH may be due to the residual charge and polar group interactions between side chains, restricted segment rotations and molecular mobility, giving rise to stiff and brittle films with high tensile strength. The results indicated that hydrophobic interactions and other non-covalent forces were responsible for stability than on disulphide bonds its native pH. The comparative decrease in the tensile property of SG-SY-G composite at pH below and above the native pH may be due to the stability conferred by disulphide bonds and, to a minor extent, by hydrophobic, covalent and hydrogen bonds. This was in agreement with earlier studies [35-36].

Table.2: Tensile strength of composite film at different pH range

Sample no	pH	Tensile strength(Mpa)	Elongation at break (%)
1	3	1.74±0.13	32.56±2.48
2	4	2.59±0.20	33.50±2.55
3	5	3.44±0.26	51.78±3.94
4	6	2.04±0.16	46.39±3.53
5	7	2.06±0.16	41.11±3.13

The data are presented as ± SD of 6 individual experiments

Total Soluble Matter:

Preheating prior to film casting had a noticeable influence on protein solubility; the higher the temperature, the lower the solubility except at the isoelectric pH (4–5). Solubility testing can yield valuable information about a film's structural properties as related to the protein treatment prior to film casting. A significant difference in degree of protein leaching at various pH with an inverse relationship with temperature was reported in earlier studies [37]. A decrease in the percentage of total soluble matter of the composite films with pH 3,4 6 and 7 (Fig. 2)with respect to the composite film at pH 5 may be due to increased protein leaching below and above the native pH of soya protein. This phenomenon may be explained in such a way that, above and below the isoelectric point (pH 5) soy proteins will carry a net negative or positive charge enhancing their solubility. The leaching of proteins from an SPI film is also an indication of weakness of protein–protein interactions in the film matrix or the strength of hydrogen bonding and ion–dipole interactions of proteins with water in the aqueous media. The relatively low protein leachability observed for the composite film at pH 5 may be due to the tight association of 7S and 11S subunits through hydrophobic interactions.

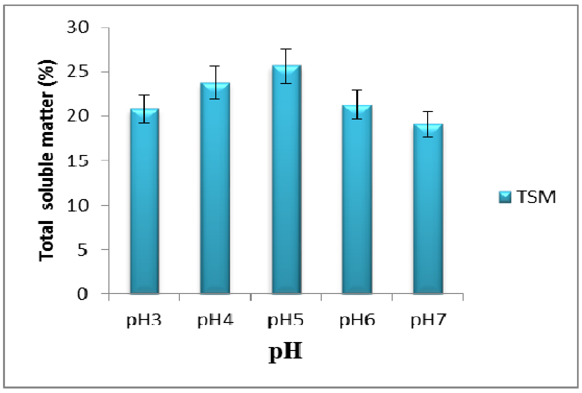

Fig.2: Total soluble matter (%) of composite film at different pH range. The data are presented as ± SD of 6 individual experiments

Moisture Content:

Although protein films are excellent oxygen barriers, generally they are of poor resistance to moisture permeation due to the predominantly hydrophilic nature of proteins [38]. In particular, SPI contains approximately 58% polar amino acids; this high polarity renders SPI films sensitive to water vapour [39]. In this study the moisture content of the film samples which differ in their pH was shown in the Table 3. The composite film with pH 5 showed higher percentage of moisture content than that of the other composite film with pH of 3, 4, 6 and 7 respectively. The moisture content of the composite film with pH 3, 4, 6 and 7 were 18%, 24%, 25% and 22% respectively. The percentage of moisture content was decreased when the pH was altered. This decrease in moisture content of the SG-SY-G composite films prepared below and above the native pH was due to the formation of covalent links in the soya protein during heating that remained in the films after casting and a reduced water solubility of film-constituting proteins due to the exposures of hydrophobic groups or the formation of hydrophobic protein aggregates [40].

Table.3: Moisture Content of composite film at different pH

Sample no	pH	Sample kept at 105^0C for 24 hours
1	3	18.2±1.39
2	4	24.9±1.90
3	5	33.4±2.54
4	6	25.3±1.93
5	7	22.1±1.68

The data are presented as ± SD of 6 individual experiments

Film Thickness:

The decreased film thickness in heated protein films was apparently due to the lower moisture content, hence, more compact and denser films. Earlier reports [41] revealed that the moisture content greatly affected the mechanical properties of soy protein "sheets". In agreement with this previous finding, composite films made in native pH showed higher moisture content, were less brittle and more elastic when compared with films prepared in the pH of 3, 4, 6 and 7 after heating (fig 4). The change in the pH showed a comparative difference in the thickness (Table 4) of the composite film samples at pH 3,4,6 and 7 with respect to the composite film formed in the native pH of 5.

Table.4: Film Thickness composite film at different pH

Sample No	pH	Thickness
1	3	0.46±0.04
2	4	0.52±0.04
3	5	0.58±0.04
4	6	0.50±0.04
5	7	0.45±0.04

The data are presented as ± SD of 6 individual experiments

Thermogravimetric Analysis:

Thermogravimetric analysis is usually recorded to determine the weight loss of the biomaterial with respect to increase in the temperature the usual end products observed in a biomaterials heated at higher temperature are NO, CO2, CO and H_2O. The stability of composite film at a higher temperature is an added advantage for biomaterials. In the present study composite films prepared at various pH ranges (3-7) were heated in a nitrogen atmosphere from 30^0 C to 750^0C. Composite film prepared in the native pH 5 (Fig 3a) showed a major between single step weight loss between 275-373^0C. The initial weight loss of 30% was due to loss of water and bound water in the sample. The thermogram of the composite film prepared in the pH range 6 (Fig 3b) showed a major single step weight loss between the temperatures 257-355^0C with a weight loss of 70%. Around 80% of weight loss was observed at a temperature 515^0C with a residue of 15% was seen at 750^0C. The thermogram of composite film with pH7 (Fig3c) showed a major single step weight loss between the temperature 248-444^0C with a weight loss 17% and 82% respectively. Almost complete loss of the sample was observed at the temperature around 650^0C with a residue of 1.2%. The thermogram of the composite film with pH5 and pH6 were comparatively stable with respect to the composite prepared at pH7. The composite films with a pH3 and pH4 were thermally unstable. The thermally unstable nature of the composite in this range (pH3 and pH4) might be due to unfolding and breaking of hydrogen bonds in the subunits of the soya protein.

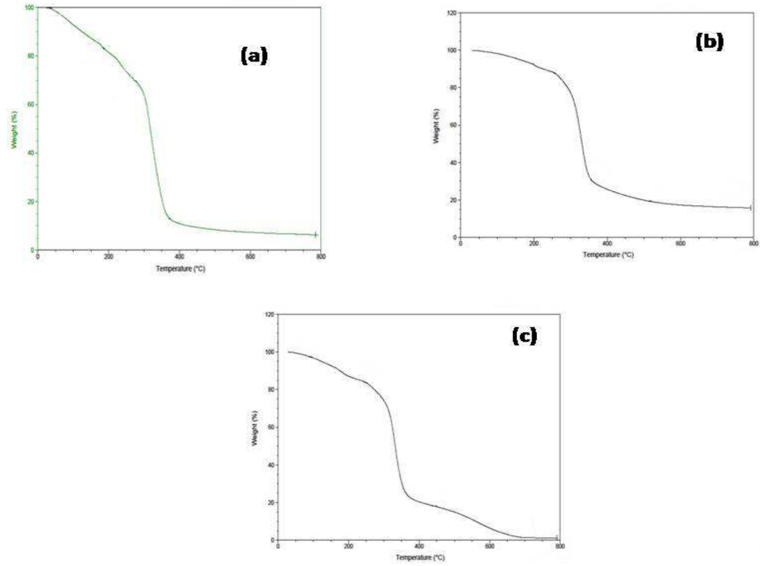

Fig.3: Thermogram of SG-SY-G film at a) pH5 b) pH6 c) pH7

Infrared Spectroscopy:

The –OH stretching vibration was observed at 3400 cm-1 for the composite films at pH 3, 4, 6, and 7. There is no predominant change in this stretching vibration. The amide I peak of SG-SY-G (pH 5) at 1633 cm-1 was shifted to 1641 and 1638 cm-1 at pH 3 and 4. In pH 6 and 7 the amide I was shifted at 1649 and 1648 cm-1. The amide II peak at 1412 cm-1 of SG-SY-G was shifted to 1435 and 1404 cm-1 at pH 3 and 4. The –OH stretching of sago starch at 1028 cm-1 (pH5) was shifted as 1088 (pH 3), 1076 (pH 4), 1065 (pH 6), 1087 cm-1 (pH 7) respectively. The C-O stretching vibration of sago starch at 873 cm -1(pH5) was shifted to 865 cm-1 at all the pH (3, 4, 6, 7). The FTIR spectral studies showed the shifting of predominant peaks when the pH of soya protein was changed from its native pH of 5. Comparing the FTIR peaks of all the films at various pH ranges, the peaks intensity was very less at pH 7 with respectively to the peaks intensity observed at pH 5 (native pH).

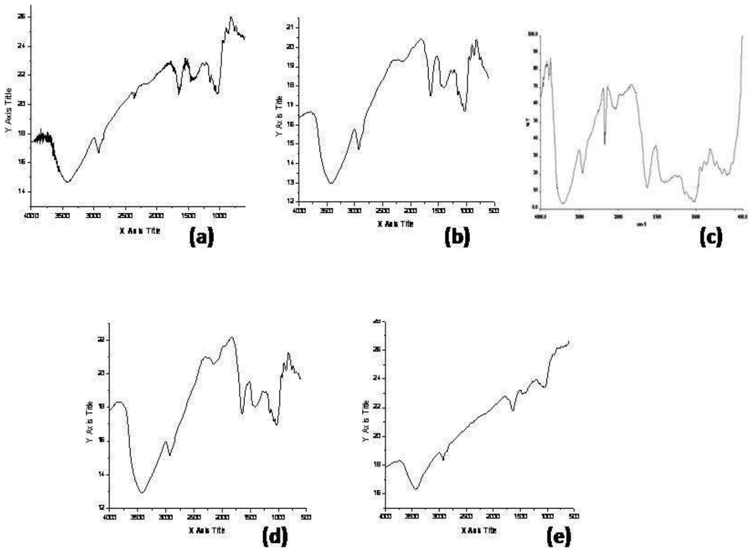

Fig.4: FTIR spectra of SG-SY-G FILM at (a) pH 3 (b) pH 4 (c) pH 5 (d) pH 6 (e) pH 7

Scanning Electron Microscope:

Scanning Electron Microscope pictures exhibit the surface morphology of biomaterial. In this study, scanning electron microscope pictures were taken for the composite film with pH 3, 4, 5, 6 and 7. The surface morphology of the composite film prepared in the pH range 3, 4, and 5 revealed smooth surfaces with porous nature. The surface morphology of the composite prepared at pH 6 and 7 also showed smooth surface with the presence of granules.

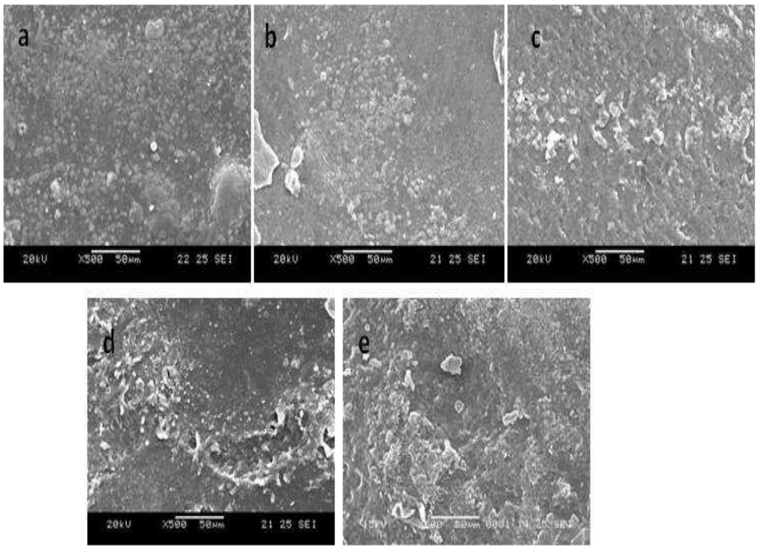

Fig.5: SEM image of SG-SY-G film at (a) pH 3 (b) pH 4 (c) pH 5 (d) pH 6 (e) pH 7

Conclusion

The composite films varying in the pH containing soya protein and sago starch cross linked with glutaraldehyde to improve their mechanical properties were investigated for its physico-chemical and mechanical properties. The tensile strength of the composite films prepared was better in the native pH compared to that of other films prepared in the pH range 3, 4, 6 and 7 respectively. This mechanical stability reveals the stable nature of soya protein in its native pH. The water absorption capacity of composite films at pH 6 showed increased water absorption after 24 hours when compared to that of other composite films. This result revealed the availability of more hydroxyl group in the polysaccharide back bone of the SG-SY-G composite films at pH 6. A decrease in the percentage of total soluble matter of the composite films with pH 3, 4 6 and 7 confirms increased protein leaching below and above the native pH of soya protein. The relatively low protein leaching observed for the composite film at pH 5 may be due to the tight association of 7S and 11S subunits through hydrophobic interactions.

The decrease in moisture content of the SG-SY-G composite films with pH 3, 4, 6 and 7 was due to the formation of covalent links in the soya protein during heating that remained in the films after casting and a reduced water solubility of film-constituting proteins due to the exposures of hydrophobic groups or the formation of hydrophobic protein aggregates. The change in the pH showed a comparative difference in the thickness of the composite film prepared. The decreased film thickness was apparently due to the lower moisture content observed in the composite films above and below the native pH of soya protein. The thermogram of the composite film with pH5 and pH6 were comparatively stable with respect to the composite prepared at pH7. The thermally unstable nature of the composite in the pH range 3 and 4 might be due to unfolding and breaking of hydrogen bonds in the subunits of the soya protein. The FTIR spectral studies showed the shifting of predominant peaks when the pH of soya protein was altered from its native pH of 5. The surface morphology of the composite prepared in the pH range 3, 4, and 5 revealed smooth surfaces with porous nature. The composite prepared at pH 6 and 7 also showed porous and smooth surfaces with the presence of granules. Overall, the films formed by pH-shifting- treated SPIs, were not superior to the film formed by native SPI in percentage elongation and tensile strength. Of particular significance, thermal stability and water absorption was slightly better above the native pH. The presented results may be useful for technological aspects since the film-forming solutions can be used in the production of biodegradable films intended for food packaging.

References

1. Santin M, Ambrosio L, Soybean-based biomaterials: preparation, properties and tissue regeneration potential, *Expert Rev Med Devices, 2008, 5(3), 349–58.*

2. Nielsen N, Structure of soy proteins. In A. Altshul, & H. Wilcke (Eds.), New proteins foods 5: Seed storage proteins, *Orlando: Academic Press. 1985a, 27–60.*

3. Nielsen N, The structure and complexity of the 11S polypeptides in soybeans*, Journal of the American Oil Chemists' Society, 1985 b,62, 1680–1686.*

4. Staswick P, Hermedson M, & Nielsen N, Identification of the cysteines which link acidic and basic components of the glycinin, *Journal of Biological Chemistry, 1984, 259, 13431–13435.*

5. Thanh V, Shibasaki K, b-conglycinin from soybean proteins. Isolation and inmunological and physicochemical of the monomeric forms. *Biochimica et Biophysica Acta, 1977, 490(x), 370–37.*

6. Kinsella JE, Functional properties of soy proteins, Journal Am.Oil *Chem. Soc., 1979, 56,* 242–258.

7. Rhim J, Gennadios A, Handa A, Weller C, & Hanna, M. Solubility, tensile, and color properties of modified soy protein isolate films, *Journal of Agricultural and Food Chemistry, 2000,48(10), 4937–4941.*

8. Gennadios A, Weller CL, Edible films and coatings from soymilk and soy protein*, Cereal Food World, 1991, 36, 1004–1009.*

9. Ghanbarzadeh B, Oromiehi AR, Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis, *International Journal of Biological Macromolecules, 2008, 43, 209–215.*

10. Wong SS, Chemistry of protein conjugation and cross-linking, *Boca Raton, FL: CRC Press; 1991,*

11. Huang-Lee LLH, Cheung DT, Nimni ME, Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks, *Journal of Bio medical Material and Research, 1990, 24(9):1185–201.*

12. Van Luyn MJA, Van Wachem PB , Olde Damink L, Dijkstra PJ, Feijen J, Nieuwenhuis P, Relations between in vitro cytotoxicity and crosslinked dermal sheep collagens*, J Biomadical Material Research, 1992;26(8):1091–110.*

13. Weadock K, Olson RM, Silver FH, Evaluation of collagen crosslinking techniques, *Artif Cells Blood Substit Immobil Biotechnol, 1983,11(4):293–318.*

14. Chen CH, Lai LS, Mechanical and water vapor barrier properties of tapioca starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer, *Food Hydrocolloids, 2008, 22, 1584–1595.*

15. Müller CMO, Laurindo JB, Yamashita F, Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films, *Food Hydrocolloids, 2009, 23, 1328–1333.*

16. Hizukuri S, Takeda Y, Usami S, Takase Y, Multi-branched nature of amylose and the action of debranching enzymes, *Carbohydrate Research, 1981,94, 205–213*.

17. Wurzburg DB, Modified starches: Properties and uses, Boca Raton, FL: CRC Press. 1986.

18. Zobel HF & Stephen AM, Food polysaccharides and their applications, New York: Marcel Dekker, 1995.

19. Moates GK, Noel TR, Parker R, Ring SG, Dynamic mechanical and dielectric characterisation of amylose–glycerol films, *Carbohydrate Polymers, 200, 44, 247–253.*

20. Arvanitoyannis I, Biliaderis CG, Ogawa H, Kawasaki N, Biodegradable films made from low density polyethylene (LDPE), rice starch and potato starch for food packaging applications: Part 1, *Carbohydrate Polymers, 1998, 36, 89–104.*

21. Arvanitoyannis I, Nakayama A, Aiba SI, Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water, *Carbohydrate Polymers, 1998,36, 105–119.*

22. Chatakanonda P, Varavinit S, Chinachoti P, Effect of crosslinking on thermal and microscopic transitions of rice starch, *Food Science and Technology, 2000, 33, 276–284.*

23. Dumoulin Y, Alex S, Szaba P, Cartilier L, Mateescu MA, Cross-linking amylose as matrix for drug controlled release. X-ray and FTIR structural analysis. *Carbohydrate Polymers, 1998, 37, 361–370.*

24. Carvalho AJF, Curvelo AAS, Agnelli JAM, A first insight on composites of thermoplastic starch and kaolin, *Carbohydrate Polymers, 2001, 45, 189–194.*

25. Wilhelma HM, Sierakowskia MR, Souzab GP, Wypychc F, Starch films reinforced with mineral clay, *Carbohydrate Polymers, 2003,52 101–110.*

26. In vivo evaluation of composite wound dressing material containing soya protein and sago starch, International journal of pharmacy and Pharmaceutical Sciences, 2012, 4 (2) 414-419.

27. Jiang J, Chen J, Xiong YL, Structural and emulsifying properties of soy protein isolate subjected to acid and alkaline pH-shifting processes, *Journal ofAgricultural and Food Chemistry, 2009, 57, 7576–7583.*

28. V Ramnath, S Sekar, S Sankar, TP Sastry, Preparation and partial characterisation of composite film containing soya protein and sago starch, *International journal of pharmacy and biological sciences, Volume 1(4), 2011,577-585.*

29. ASTM Standard test method for tensile properties of thin plastic sheeting. In Annual book of ASTM standards Designation D 882-95a. Philadelphia, PA: *American Society for Testing and Materials, 1997, 159–167.*

30. Xu JB, Bartley JP, Johnson RA, Preparation and characterization of alginate–carrageenan hydrogel films crosslinked using a water-soluble carbodiimide (WSC), *Journal of Membrane Science, 2003, 218.*

31. Jiang J, Chen J, Xiong YL, Structural and emulsifying properties of soy protein isolate subjected to acid and alkaline pH-shifting processes, *Journal of Agricultural and Food Chemistry, 2009,57, 7576–7583.*

32. Jiang J, Xiong YL, Chen J, PH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions, *Journal of Agricultural and Food Chemistry, 2010,58, 8035–8044.*

33. Pace CN, Polar group burial contributes more to protein stability than nonpolar group burial, *Biochemistry, 2001, 40, 310–313.*

34. German B, Damodaran S, Kinsella JE, Thermal dissociation and association behavior of soy proteins, *Journal of Agricultural and Food Chemistry, 1982,30, 807–811.*

35. Rangavajhyala N, Ghorpade VM, Hanna M, Solubility and molecular properties of heat-cured soy protein films, *Journal of Agricultural and Food Chemistry, 1997, 45, 4204–4208.*

36. Rhim JW, Gennadios A, Handa A, Weller CL, Hanna MA, Solubility, tensile and color properties of modified soy protein isolate films, *Journal of Agricultural and Food Chemistry, 2000, 48, 4937–4941.*

37. Jiang Jiang, Youling L, Xiong, Melissa C, Newmanb Gregg K, Rentfrow, Structure- modifying alkaline and acidic pH-shifting processes promote film formation of soy proteins, *Food Chemistry, 2012. 132 1944–1950.*

38. Krochta J, Mulder-Johnston CD, Edible and biodegradable polymer films: Challenges and opportunities, *Food Technology, 1997, 51, 61–74.*

39. Rhim JW, Lee JH, Effect of CaCl2 treatment on mechanical and moisture barrier properties of sodium alginate and soy protein-based films, *Food Science and Biotechnology, 2004,13, 728–732.*

40. Jiang J, Xiong YL, Chen J, PH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions, *Journal of Agricultural and Food Chemistry, 2010),58, 8035–8044.*

41. Zhang J, Mungara P, & Jane J, Mechanical and thermal properties of extruded soy protein sheets, *Polymer, 2001, 42, 2569–2578.*

Source of support: Nil
Conflict of interest: None Declared