Mechanical and Thermal Properties of a Novel Protein-Based Plastic Synthesized Using Subcritical Water Technology

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ABSTRACT: This work reports on the characterization of a novel protein-based plastic synthesized from bovine serum albumin (BSA) using subcritical water technology. In the present study, the mechanical and thermal properties of the novel plastic (PBSA) are presented while investigating the effects of blending biodegradable organic compounds on its mechanical properties. The average ultimate tensile strength (UTS) of the processed PBSA samples was 12.7 MPa with a modulus of elasticity (E) and tensile strain at the break (ϵ) of 370 MPa and 1.7%, respectively. Blending 10% glycerin with PBSA resulted in a reduction in the UTS (10.1 MPa) but increased the ϵ value (4.4%). An increase of the glycerin percent to 25% further decreases the UTS (8 MPa) while further increasing ϵ (7.8%). The addition of 10% starch resulted in a reduction in both the UTS and ϵ (7.3 MPa, 1.3%). Conversely, the PBSA with 25% starch blends showed a dramatic reduction in UTS (0.6 MPa) while increasing the ϵ value (2.8%). A scanning electron microscope (SEM) was used to investigate the morphological properties of the tested samples. The SEM images showed a smooth and homogeneous morphology in the glycerin blend and a phase separation in the starch blend.

Introduction

The use of petroleum-based plastic has resulted in severe environmental contaminants due to waste disposal problems.[1] Moreover, no petroleum-based plastic is sustainable and we must move quickly to produce economically feasible materials, which should be renewable with having a high performance. As for the plastics economy, bio-based and biodegradable plastics hold out the most promise for the future. The term biodegradable means that a substance is able to be broken down into simpler substances by the activities of living organisms, and therefore is unlikely to persist in the environment. The concept of combining biodegradability with the high availability of natural polymers is attracting scientists from diverse areas. Recently, protein-based materials (from vegetable or animal origin) are proposed as a potential solution to provide a new biomaterial as well as biodegradable plastic.[2] Proteins are very versatile materials, both by source and because they provide a wide variety scale of possible modifications. Accordingly, they can vary from being rigid to flexible in structure and have similar mechanical properties as do existing polymers with features that can be tailored toward the diverse requirements of a specific application. Furthermore, proteins are similar to tissue constituents, which may lead to the introduction of novel biomaterials into the green plastic field or clinical use.

For all above-mentioned properties and capabilities, proteins may be regarded as an eventual ideal template suitable for being used as biomaterials. Collagen and its denatured form gelatin (obtained by the hydrolysis of collagen) are famous examples of protein-based biodegradable plastics, which have been extensively studied and used as a biodegradable plastic in different fields.[3,4] In addition to collagen, other proteins have been used in the production of biodegradable plastics. Examples of these proteins include casein,[2] soy protein,[5] and zein (a family of proteins found in corn and derived by solvent extraction).[6,7] Accordingly, the introduction of other new protein-based biomaterials may be of extreme importance for extending and developing the biodegradable plastic field. Vaz and others give two important features that a new protein-based plastic should possess to be qualified as an acceptable biodegradable plastic.[2] First, such a material should have a reduced susceptibility to thermal degradation (allowing for its easy processing by melt-based technologies into complex 3D structure), and second, it should display convenient degradation behavior.

Serum albumin represents the most abundant protein in blood serum with a typical concentration of 50 g/L. The word albumin is also used to describe a protein or a group of proteins defined by their solubility in water such as those found in the albumin fraction of wheat and serum albumin of proteins in whey-milk.[8] Typically, bovine serum albumin (BSA) is a monomeric highly water-soluble protein with a molecular weight of 66 kDa. In our previous work,[9] using the BSA, we succeeded in synthesizing a novel biodegradable plastic (PBSA). The synthesis processes were carried out using subcritical water in batch reactors within a very short reaction time (1 min) at 250 °C. The polymerization of the BSA was catalyzed under the subcritical water condition without any additives (the present authors provided more details for the efficiency of using the subcritical water as a reaction media).[10] Both the simulation of the reaction kinetics and synthesis mechanism of the PBSA were investigated in two different studies.[11,12]

On the basis of the promising results obtained in our previous work, it was necessary to go even further to evaluate the different properties of the PBSA to confirm its efficiency as a new product as well as exploring its applicability in the different plastic related fields such as new implant material.

In the present work, a more detailed analysis of the mechanical and thermal properties of PBSA are presented while investigating the effects of blending biodegradable organic compounds over the mechanical performance of PBSA. Then, the biodegradability behavior was also investigated.

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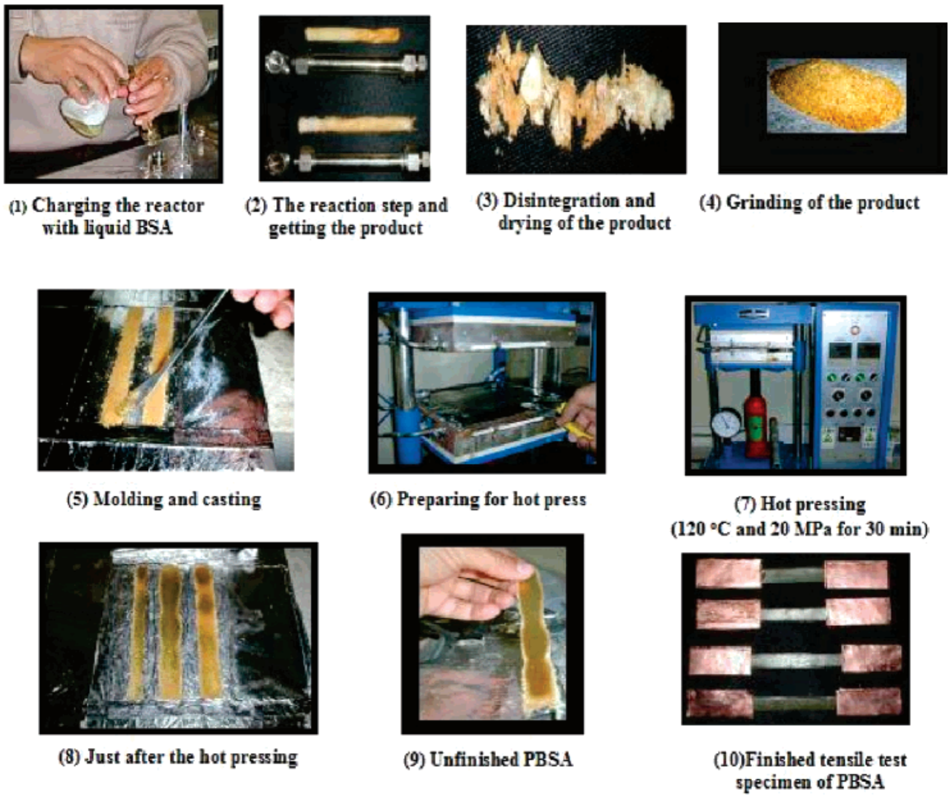


(1) Charging the reactor with liquid BSA
(2) The reaction step and getting the product
(3) Disintegration and drying of the product
(4) Grinding of the product
(5) Molding and casting
(6) Preparing for hot press
(7) Hot pressing (120 °C and 20 MPa for 30 min)
(8) Just after the hot pressing
(9) Unfinished PBSA
(10)Finished tensile test specimen of PBSA

Figure 1. The processing steps of PBSA synthesized using subcritical water technology.

Materials and Methods

Materials. Pure BSA and other chemicals used in this study were produced by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The BSA used was found to be homogeneous in the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis with 5% water content.

Synthesis of the Biodegradable Plastic. The synthesis was carried out according to the method described in our previous work.[9] Briefly, the PBSA was prepared by reacting a solution of BSA under the subcritical water condition. The synthesis process was carried out in a batch reactor at 250 °C for a reaction time of 1 min using 160 g/L BSA as an initial concentration.

Processing of the PBSA. The different processing steps of the PBSA are shown in Figure 1. The solid product (raw PBSA) resulted from the reaction of BSA under the subcritical water condition and was recovered from the reactor in the form of a long cylindrical bar and dried at 50 °C for 24 h. Then, the dried PBSA was ground into powder form using a TML-16 grinder (TESCOM Co., Tokyo, Japan). Solid and dried PBSA in powder form were hand mixed and stirred with water until a homogeneous slurry mixture was obtained. The percentage of water in all experiments was kept constant at 150% based on the mass of the solid PBSA. The final mixture was then poured into a test specimen mold made from aluminum foil mounted over a stainless steel plate. Then the mold was covered with another steel plate covered with aluminum foil. The stainless steel plates containing the test specimen in between were then subjected to a hot pressing using a hot pressing machine equipped with a thermostat (to control the temperature of the pressing) and pressure gauge (for monitoring the pressure). The hot pressing was carried out at 120 °C and 20 MPa for 30 min. Then, the mold was removed from the hot pressing machine and allowed to cool down to room temperature. The test specimen was removed and adjusted to a definite shape and dimension using a sharp cutter. Two strips of braces were used to clamp the ends of the specimen to avoid over stress during the tensile test. The same procedures were followed in the case of PBSA blends. The water content was also kept constant at 150% based on the total solid-blend content throughout all experiments.

Electron Microscopy. A JEOL 6700F field emission scanning electron microscope (SEM) (JEOL Inc, MA) was used to observe the microstructure of the prepared PBSA. Solid dry samples of the PBSA were prepared for the SEM according to the manufacturer's instructions.

Differential Thermal Analysis. Thermal properties of the PBSA material and its blends were studied using a differential thermal analyzer (Rigaku TG-DTA, TAS-100, Rigaku Co., Tokyo, Japan). The measurements were carried out in air with a heating rate of 10 °C/min following the manufacture's standard procedures. Data acquisition and analysis software were provided by the instrument manufacturer. According to the manufacturer's measuring procedures, alumina was used as a reference material.

Mechanical Properties of the PBSA. Both processed and blended PBSA specimens were tested for tension at room temperature using an Instron 5582 testing machine (Instron Corp., MA) equipped with 100 kN static load cell. The gauge length of the test machine was set at 40 mm. The samples were clamped so that the interface was equidistant from each clamp. All samples were pulled at a rate of 10 mm/min and were evaluated for the average ultimate tensile strength (UTS), the strain at break (ϵ), and the modulus of elasticity (E) in the interval of 0.05−0.25 strain.

Biodegradability Test. The biodegradability test was carried out in accordance with the method described elsewhere.[2] Three molded specimens (one batch) of PBSA weighing approximately 1.3 g each was aged up to 120 days in an isotonic saline solution (ISS; NaCl 0.154 M, pH 7.4) at 37 °C. Both water uptake and sample weight losses were measured to identify both the water absorption and degradation rates.

Results and Discussions

Mechanical Properties. The first step of this study was to synthesize and process of our new protein-based plastic. Figure 1 shows both the synthesis and processing steps. The synthesis steps were discussed in detailed in our previous work.[9] The processing steps were first optimized by a trial and error routine, since the product is new and there was no similar product in

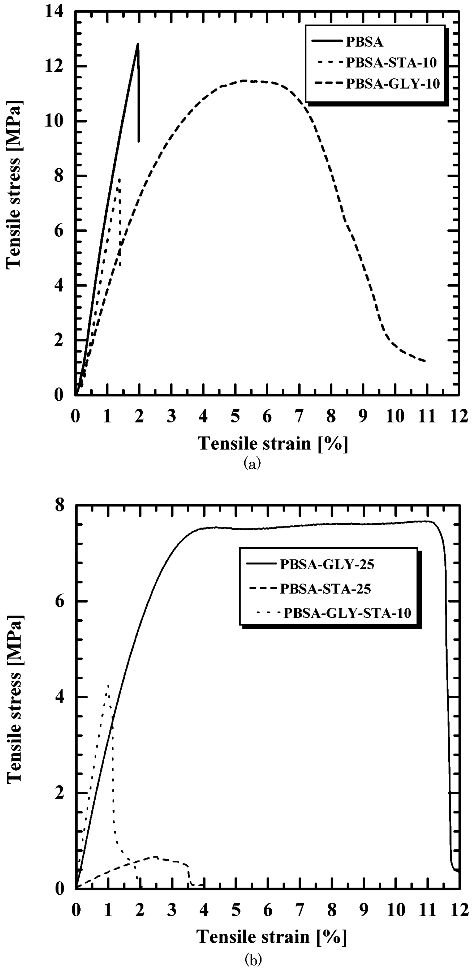


Figure 2. The stress–strain curve of (a) the native processed PBSA and PBSA blended with 10% glycerin or starch and (b) PBSA blended with 25% glycerin, or starch and PBSA blended with a mixture of 10% of both starch and glycerin.

Table 1. The Mechanical Properties of the PBSA and Its Blends

sample	PBSA [%]	GLY [%]	STA [%]	UTS [MPa]	ϵ [%]	E [MPa]
PBSA	100	0	0	12.9	1.7	370
PBSA-GLY-10	90	10	0	11.1	4.9	240
PBSA-GLY-25	75	25	0	8	7.8	309
PBSA-STA-10	90	0	10	7.3	1.3	170
PBSA-STA-25	75	0	25	0.6	2.8	50
PBSA-STA-GLY-10	80	10	10	6	1.2	538

the market to be taken as a guide. After many trials we could adapt a simple and straight forward strategy for the processing methodology. Then, the processed plastic was used for evaluating the different mechanical, thermal, and biodegradability properties.

The mechanical properties of the native processed PBSA samples were compared with those blended with glycerin, starch, or a mixture of both starch and glycerin. The detailed steps of the processing are described in the Materials and Methods section. For most materials, a simple tensile stress–strain curve provides a good start toward understanding the mechanical behavior of a particular plastic or polymer. This curve is usually established by continuously measuring the force developed as the sample is elongated at a constant rate of extension until it breaks. Figure 2 shows the stress–strain curves of the tested samples while Table 1 lists their mechanical properties. The native processed PBSA and PBSA blended with 10% glycerin or starch are shown in Figure 2a. For the native PBSA samples, the average values of UTS, E, and ϵ were 12.9 MPa, 370 MPa, and 1.7%, respectively. The tested native PBSA samples showed a typical stress–strain curve to those of brittle materials which are linear over their full range of strain while exhibiting elastic deformation up to the point of fracture without appreciable plastic flow. This indicates that such materials are lacking mobility which indicates that they have internal microstructures that block dislocation motion. Accordingly this result signifies that the native PBSA samples are brittle rather than ductile.

To study the effect of adding a plasticizer, PBSA samples were plasticized with glycerin (also called glycerol). Glycerin is an inexpensive and effective plasticizer, which tends to make the resulting material more plastic and flexible even at a very low temperature. The results showed that blending the PBSA with 10% glycerin resulted in a reduction in the UTS to about 14%; however, at 25% glycerin the UTS reduced more to about 38%. The modulus of elasticity, E, showed a reduction of about 35% at 10% glycerin indicating a reduction in the stiffness of the sample; however, by increasing the glycerin percentage to 25%, the stiffness increased when compared to the 10% glycerin samples but was still lower than that of the native PBSA samples by about 16%. Moreover, the results showed that the tensile strain and the area under the stress–strain curve (which represents the work needed to break) increases as the glycerin percentage increases. These results indicate that adding glycerin to the native PBSA samples resulted in increasing the mechanical plasticity and transforming the PBSA into a tougher and softer material. This simply means that PBSA became a ductile material capable of sustaining large plastic deformations before fracture.

The mechanism by which glycerin increases the toughness and softness is still not completely understood. The observed increase of mechanical plasticity is pictured as arising from that glycerin may form cross-links with the PBSA molecules leading to a decrease in mobility, but at the same time some formed glycerin clusters increase the chain mobility by spacing out the chains and therefore increase the plasticity.[13]

To study the effect of blending PBSA with the starch on the mechanical properties, potato starch was blended with PBSA with different weight percentages. In general, starch was found to deteriorate the mechanical properties of PBSA. The results showed that at 10% starch, the UTS, E, and ϵ values were reduced by about 43%, 54%, and 23%, respectively. Conversely, PBSA with 25% starch blends showed a dramatic reduction in the strength and stiffness of the PBSA samples since the results showed that the UTS and E were reduced by about 96% and 87%, respectively. However, an increase in the ϵ value by about 64% was observed.

To get compromised mechanical properties, 10% of both glycerin and starch were blended with the PBSA material. The results showed that the stiffness was highly improved which was indicated by the dramatic increase in the E value to almost twice that for the native PBSA. Conversely, both UTS and ϵ values were reduced by about 53% and 29%, respectively. These results indicate a reduction in hardiness, toughness, and in turn, flexibility of such a blend compared to the native PBSA.

Microstructure. The micrographs from the SEM of the BSA, raw PBSA (before processing), and the processed PBSA are shown in Figure 3. The micrograph of the BSA (Figure 3a**)** showed a smooth surface morphology while the raw PBSA (Figure 3b**)** showed groups of aggregated BSA molecules which differed in their cluster size. The raw PBSA also showed a strong network preventing the mobility of the molecules and resulting in blocking the dislocation motion. Such a strong structure

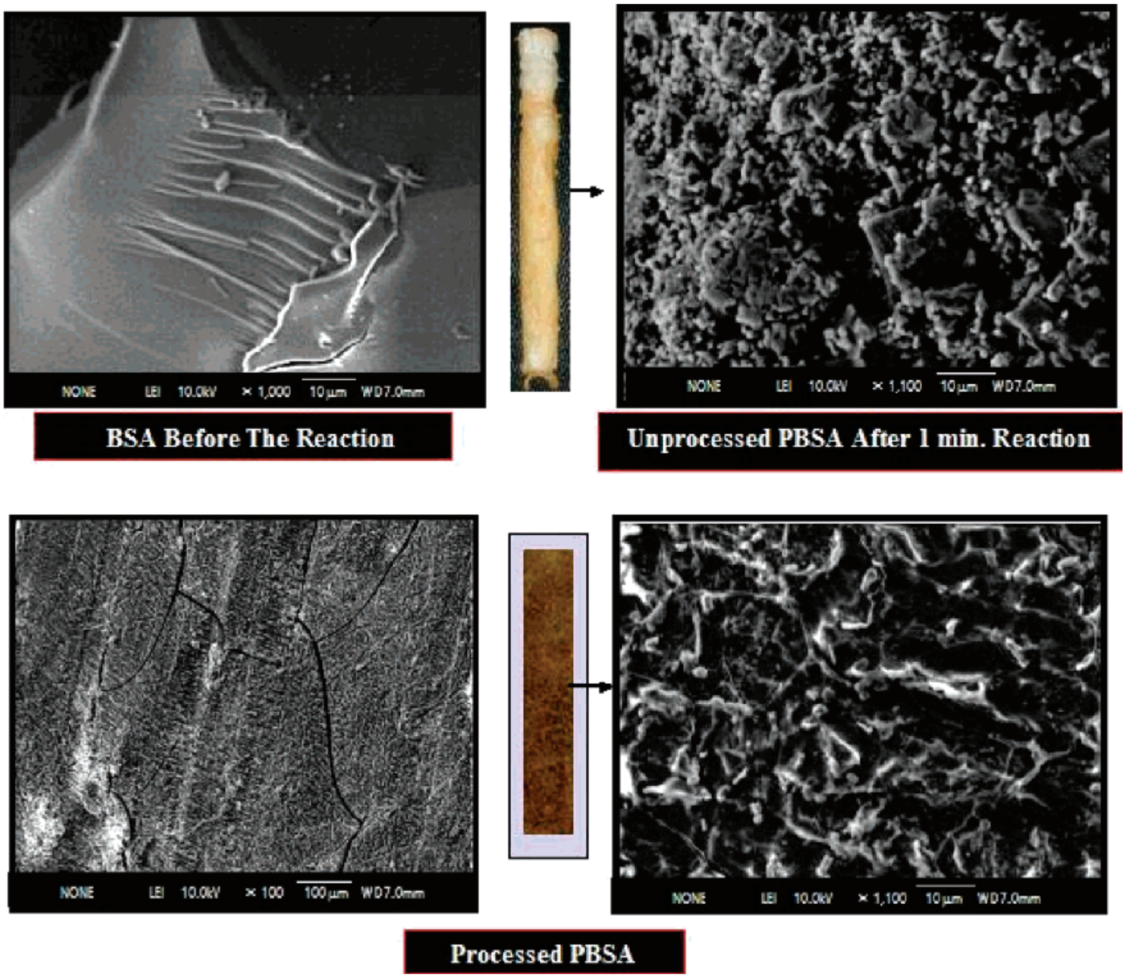


Figure 3. The SEM micrograph of the (a) BSA, (b) unprocessed PBSA, and processed PBSA at (c) at 100× and (d) 1100×.

appeared clearly in the micrograph of the processed PBSA (Figure 3c,d**)** which showed protein fibrils with a strong interconnected network structure. However, the processed PBSA still preserved small agglomerations of the original raw PBSA clusters. Such a strong network was responsible for enhancing the material stiffness while the clusters still hindering the dislocation motion resulted in reduced flexibility.

Figure 4 shows the microstructure images produced by the EMS of the fracture surface of the PBSA blends at a low magnification of 100× (on the left side) and a high magnification of 1100× (on the right side).

EMS images of the 10% glycerin blend revealed that glycerin swept up the cluster's agglomerations of the native PBSA (Figure 4a,b). Such an effect explained the increased in toughness of such a blend over the native PBSA. On the contrary, the EMS of the 10% starch blend showed a phase separation between the PBSA and starch and the development of starch aggregates (Figure 4c,d). These aggregates are clearly observed when the starch percentage was increased to 25% **(**Figure 4e,f).

The blending of 10% of both starch and glycerin resulted in a compromised structure **(**Figure 4 g,h). The glycerin worked as a homogenizer and reduced the phase separation between starch and the PBSA as well as preventing starch aggregations.

Thermal Analysis. The differential thermal analysis (DTA) is considered to be a "fingerprinting" technique that provides information on the chemical reactions, phase transformations, and structural changes that occur in a sample during a heat-up or a cool-down cycle. The DTA measures the differences in energies released or absorbed as a function of temperature. This is done by measuring the difference in temperature between a sample and a thermally inert reference as the temperature is raised. The plot of this differential provides information on exothermic and endothermic reactions taking place in the sample.

In this case, the target of this experiment was to evaluate the relative magnitude of reactions and phase transitions which may have took place between the PBSA and both the starch and glycerin as well as estimating the relative thermal stability of these novel materials. From that information, safe drying and processing schemes can be determined.

Figures 5 and 6 show the DTA thermograms obtained for the raw solid BSA, unprocessed PBSA, processed PBSA, and PBSA blends, respectively. The thermogram parameters for all tested samples were obtained from these figures and are listed in Table 2. These parameters include initial (Tx1) and second (Tx2) decomposition temperatures as well as the late charring temperature (Tx3). The Tx1 and Tx2 values for the raw BSA were lower than that of the unprocessed PBSA. The increase of the initial and second decomposition values were perhaps due to cross-linking reactions which took place during the synthesis of the unprocessed PBSA from the raw BSA under the subcritical water condition (for detailed synthesis steps refer to our previous work).[8] However, the Tx1 for the processed PBSA was found to be less than those of the raw BSA and the unprocessed PBSA. The reason behind this lower value is due to the plasticizing effect of the added water during the processing of the PBSA. Blending the PBSA with 10% glycerin or 10%

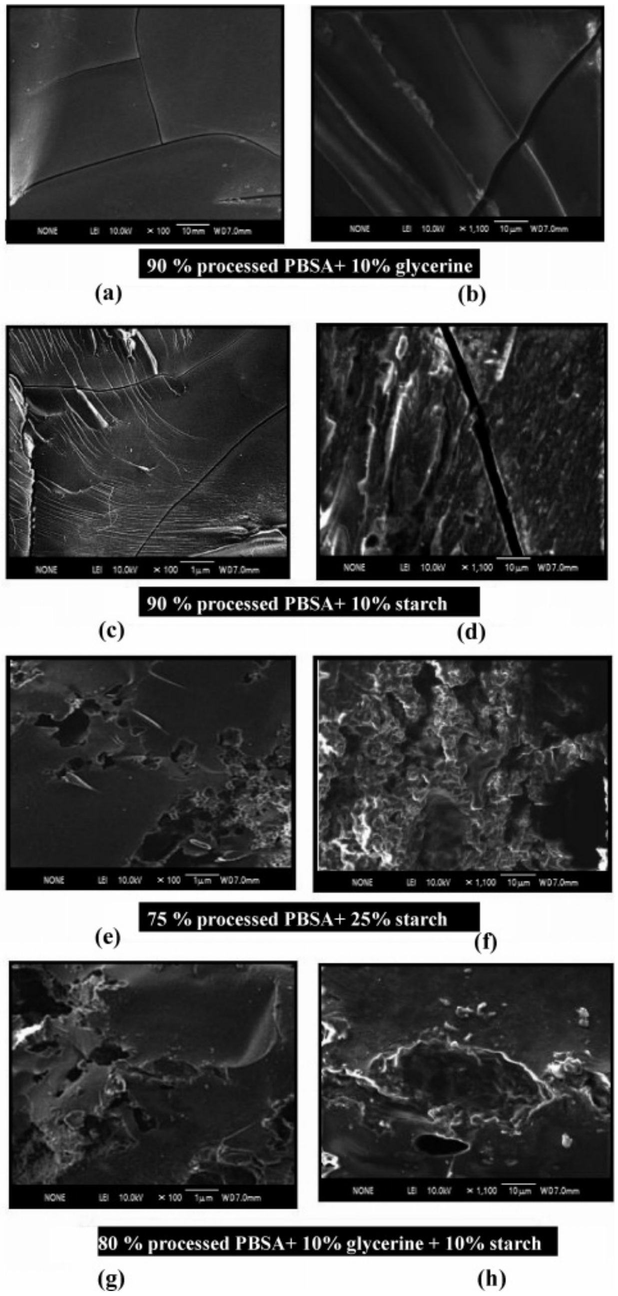


Figure 4. The SEM micrograph of the PBSA blends at different levels of magnification 100× (on the left) and 1100× (on the right), respectively. (a and b) 90% Processed PBSA and 10% glycerin, (b and c) 90% processed PBSA and 10% starch, (d and e) 75% processed PBSA and 25% starch, and (g and h) 80% processed PBSA, 10% glycerin, and 10% starch.

starch or a mixture of both resulted in enhancing the thermal stability indicated by the increase of the Tx1 value. Moreover, the thermogram of the PBSA blended with a mixture of both 10% starch and 10% glycerin showed Tx1 values at 317 °C and Tx2 at 342 °C due to the difference in the first decomposition temperatures of both glycerin and starch. Such behavior was not observed in the case that starch or glycerin was used alone, which may signify the occurrence of some cross-linking reactions.

The temperatures of the late decomposition were almost similar in all tested samples. All were in the range of the late decomposition temperatures of protein and organic compounds.

Thermogravimetric analysis (TGA) measures changes in weight of a sample while increasing the temperature. TGA provides a quantitative measurement of any mass change in the polymer or materials associated with a transition or thermal degradation. It can also record the change in mass due to dehydration, decomposition, or oxidation processes in a given time and temperature. TGA curves are therefore characteristic for a given polymer or compound because of the unique sequence of the physicochemical reaction that occurs over specific temperature ranges and heating rates and are a function of the molecular structure. The changes in mass result from the rupture and/or formation of various chemical and physical bonds

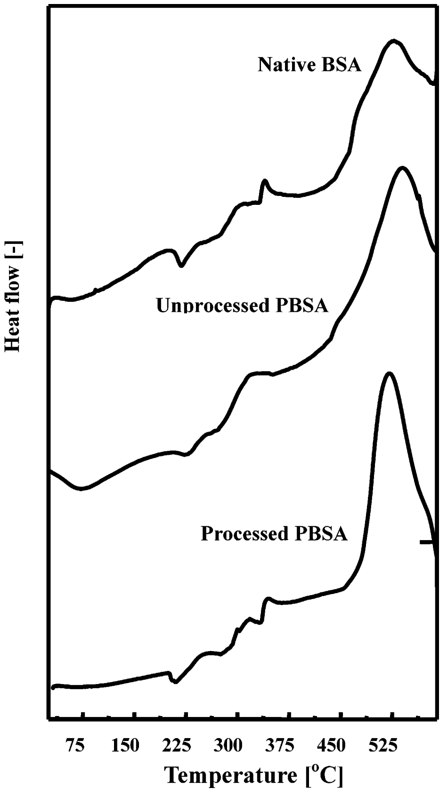


Figure 5. DTA thermographs of the raw solid BSA, unprocessed PBSA, and processed PBSA.

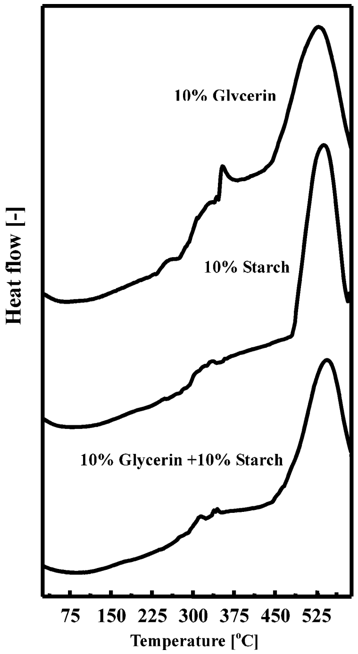
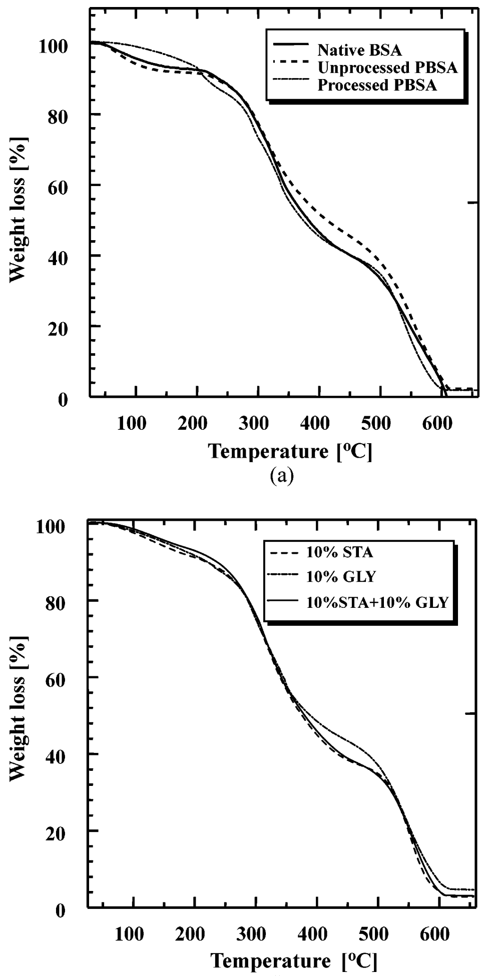


Figure 6. DTA thermographs of PBSA blended with 10% glycerin or 10% starch or a mixture of both.



(a)

(b)

Figure 7. GTA thermographs of (a) the raw BSA, unprocessed PBSA, processed PBSA and (b) PBSA blended with 10% glycerin or 10% starch or a mixture of both.

Table 2. The DTA Parameters of Raw BSA, Unprocessed PBSA, Processed PBSA, and Different PBSA Blends

sample	Tx1 [°C]	Tx2 [°C]	Tx3 [°C]
native BSA	216	339	526
unprocessed PBSA	228	345	540
processed PBSA	210	316	520
PBSA-GLY-10	235	354	529
PBSA-STA-10	300	338	538
PBSA-STA-GLY-10	317	342	546

at elevated temperatures that lead to the evolution of volatile products or the formation of heavier reaction products.

Figure 7 shows the TGA curves for all tested samples. The TGA results confirmed the data obtained by the DTA. The obtained weight loss patterns were compatible with the decomposition temperatures obtained by the DTA. In general, TGA thermograms of the tested samples had two ramps of weight loss. The raw BSA, unprocessed PBSA, and processed PBSA had these ramps at 203 °C and 460 °C. TGA thermograms of the PBSA blended with 10% glycerin or 10% starch or a mixture of them both showed weight losses at 250 °C and 480 °C, signifying enhancement of the thermal stability compared to the PBSA samples.

Water Absorption and Degradation Rate. The biodegradation of the PBSA in fermentation media was proved and presented in detail in our previous work.[8] The results showed that PBSA samples incubated for one week and 1 month in fermentation media lost 15% and 35% of their weight, respectively, due to biodegradation reactions. However, in this work a type of working biodegradability test was adapted to show if the new material's degradation behavior was convenient to fulfill the second feature stated by Vaz and others[2] for a given biodegradable plastic to be acceptable. Moreover this test shows the water absorption ability of such a new material.

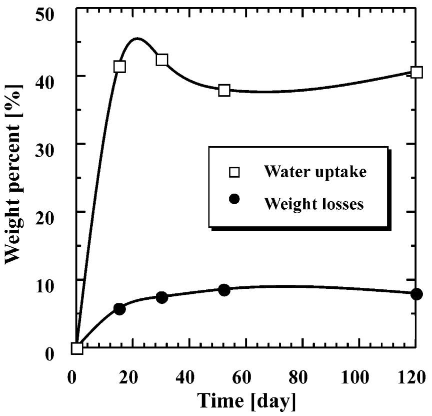


Figure 8. The time course of the water uptake and weight losses of aged PBSA samples in ISS.

Figure 8 shows the water uptake and weight losses of aged PBSA samples in ISS for a period of 3 months. The results showed that the rate of weight losses was considerably high during the first month but minimal weight losses were observed in the subsequent 2 months. The maximum recorded weight loss was less than 10% (approximately 8%). Such results revealed that the PBSA could be considered a stable compound under the tested condition with high durability from the degradation point of view. For water absorption, the rate was high at the beginning but with no further absorption after almost 20 days with a maximum water absorption of 45%. Some water desorption was observed after 1 month due to the degradation reaction which took place during that period.

Conclusions

This preliminary study confirms that the newly developed protein-based plastic, PBSA, displays a suitable range of mechanical, thermal, and degradation properties. This might eventually allow its use in the traditional application of bioplastic as well as an implanted biomaterial in the clinical area. For many applications it will be necessary to enhance and improve its mechanical properties by testing the effect of a wide range of plasticizers and other filling materials on its mechanical and biodegradation properties.

In the near future we plan to present a more efficient way of blending PBSA with other organic materials for the sake of modifying the properties using subcritical water technology.

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