Fabrication of Biopolymer Wound Dressing by Solvent Casting Technique

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Abstract

Biopolymers and their applications have been widely studied in recent years. Current wound dressings have disadvantages such as less flexibility, poor mechanical strength, lack of porosity, and a tendency for dressings to adhere onto the wound surface. In this work, two biomaterials i.e. alginate and soy protein isolate (SPI) were used to fabricate biopolymer wound dressing. The aim of this study is to investigate the effect of alginate and soy protein ratio on the chemical and physical properties of the hybrid biopolymer wound dressing and to determine effect of addition glycerol into the fabricated alginate/soy protein wound dressing. The biopolymers were characterized by its physical and chemical analysis such as chemical composition, porosity, surface morphology, thermal degradation using FTIR, SEM, and DSC and TGA, respectively. The alginate weight ratio was ranging between 1, 2% and 3 wt% and 0.5, 1.0 and 1.5 wt% of soy protein ratio, respectively. The synthesized biopolymers were dried by supercritical drying using CO_2 at 120 ± 5 bar and 40 ºC to remove excess solvent from the hybrid biopolymer wound dressing. Results demonstrated that increase in the alginate content increased the roughness of the biopolymer films whereas the SPI content promotes smother surface onto the film. The addition of glycerol had increased the mechanical strength of the biopolymer film. This study suggests that the characteristics of the biopolymer can be improved by addition of another type plasticizer and different biomaterials to improve the surface morphology of the biofilms as well as thermal stability.

Keywords: *Wound Dressing; Alginate; Soy Protein Isolate; Biopolymer; Glycerol*

1. Introduction

Acute and chronic wounds need a proper wound care management however its face many limitations in the research and medical field. Acute wound heals faster than chronic wound such as traumatic and surgical wound but having the limitation for the wound care such as high cost and painful process. Biomaterials as wound dressing have the ability to accelerate the healing phase in term of migration and proliferation. However, a biomaterial alone is not sufficient to provide adequate healing ability for the wound. Thus, addition of various type of chemical element or combining two or more biomaterials with additional features such as cross-linking can be a good strategy to produce a good biopolymer wound dressing.

Skin damage such as accidental burn by flames or touching hot water. These kinds of accidents may come with high cost of treatment of even may lead to death, [1]. Biodegradable polymers have become attraction in the field of biomaterials and tissue engineering due to its ability to prevent any additional surgery process such as removing scaffold or implants in body [2].

the wound to stimulate the cell growth. Good antiseptic properties, mechanical strength and natural material ingredients are characteristic of good wound dressings, [4]. In order to protect the wound against microorganism, it must be permeable for moisture and oxygen as physical barriers, [5]. Natural polymers are good choice to be used and prepared different kind of wound dressing such as alginate, collagen and cellulose but there still lacking in some features, [6].

Development of new biodegradable materials have been increasing for use in wound care application such as soy protein isolate at which is very promising natural materials and many benefits it contains,[7]. The conventional wound dressing such bandage of gauze is impermeable to bacteria and liquid but permeable to moisture vapor and air and the wound fluid may accumulate underneath the film because these kinds of dressings are non-absorbent which is a major disadvantage of such dressings, [8]. According to [9], an ideal wound dressing must be able to do the healing process by maintain the moist environment at the wound site to prevent excess exudate and stimulate cell growth, can be detach easily and less painful

to the injuries, reduce scarring while allowing the oxygen permeability to the wound site. The wound dressing must exhibit the ability to protect the wound site against bacterial invasion and be low adherent to the wound to make it easier to be remove after healing. The wound dressing also must be non-toxic, non-allergic and biodegradable [10].

Microbial activity can cause exudate formation, slowing the wound healing rate and can result in scar or even threaten the life of the patience, [11]. The main attention of the alginate based wound dressing is that its ability to absorb exudates up to 20 times however the alginate base dressing have no adhesive properties which is essential to maintain the moist environment on the wound site and very porous at which come a combination of another biopolymers to overcome the desired properties for the wound healing phase [5].

The development of materials that possess biodegradability from renewable biopolymers has increase remarkably such as soy protein isolate due to its nutritional efficacy and functional at which it can be obtain at low price for biomedical application,[7]. However, soy protein isolate is soluble in water and may fail to form water resistance wound dressing. As for burned patients, nutritional support such has special omega-3 fatty acids, amino acids, which are glutamine and arginine is one of the most important aspect in wound care management [12].

Addition of glycerol as plasticizer resulted in a significant decrease in the rigidity, decrease of the tensile strength and an increase in the elongation at break of the wound dressing, [13]. Glycerol can affect the structure of the polymer chain resulting in increase of crystallinity and make the composite biopolymer film softer but high in term of flexibility, [14]. Calcium ion have been reported as cross linker in many studies for the preparation of the alginate film thus calcium chloride is use as cross linker in the preparation of the hybrid biopolymer wound dressing, [15].

The hybrid biopolymer dressing was synthesized by solvent casting technique and dried by supercritical CO_2 drying. The effect of the alginate/soy protein ratio on the physical and chemical properties of the wound dressings were which including porosity, chemical composition, thermal characterization, surface morphology, water uptake and swelling ratio and mechanical tensile strength. Effect of the addition of glycerol on the characteristics of the wound dressing was investigated. Specifically, this study aims to investigate the effect of ratio alginate/soy protein on the chemical and physical properties of the hybrid biopolymer wound dressing effect of the antibacterial activity of fabricated alginate/soy protein wound dressing.

2. Material and methods

2.1 Materials

Sodium salt alginic acid, absolute ethanol and calcium chloride were purchased from Merck. Soy protein isolate (99% purity) was purchased from Xi'an Sky Biological Technology Co., Ltd., China.

2.2 Synthesis of Alginate-Soy Protein Isolate Hybrid Biopolymer

There were 9 set of samples was prepared by mixing different amount of biopolymer. The alginate fraction was set between 1 and 3 wt% of the 100 ml distilled water beaker consisting of 6 samples with different percentage of soy protein isolate and glycerol. The glycerol was added prior to 50 wt% of the weight of alginate powder added into the solution. Three samples were prepared with 3 wt% of alginates ratios with 0.5, 1.0 and 1.5wt% of soy protein fraction, respectively, inside 100 ml of distilled water without addition of glycerol. These sample were considered as blank samples.

The series of mixture of 1% and 3% alginate concentration were stirred at room of conditions of 400 rpm for 2-3 hours. The series of resulting mixture were poured into petri dishes until a thin layer of film formed and were labelled as AG/SPI-1, AG/SPI-2, AG/SPI-3, AG/SPI-4, AG/SPI-5, AG/SPI-6, AG/SPI-7, AG/SPI-8 and AG/SPI-9 respectively. The $CaCl_2$ was added drop by drop to the series of petri dish. The films then were undergone supercritical drying by CO_2 at 120 ± 5 bar and 40 ^oC to remove excess solvent from the hybrid biopolymer wound dressing.

2.3 Biopolymer Characterization

Scanning Electron Microscopy JEOL JSM 820 model Bruker Quantax 2000 was used to measure the morphology of the synthesized biopolymer at high resolution images at voltage of 2-4kV. Prior to the analysis, the samples were splattered-coated with gold layer. The soaked biopolymer films were qualitatively characterized by FTIR (Bruker Platinum-ATR) equipped with software of OPUS Optik GmBH in the range from 400 to 4400 cm^{-1} of wavelength.

Thermal characteristics of the hybrid biopolymer wound dressing was determined using TGA Mettler Toledo SAE system. The analysis were carried out from 20 to 600 ^oC at rate of 10 ^oC/min. Meanwhile for the differential scanning calorimetry (DSC) (Model Mettler Toledo) was analyzed at 25 ^oC to 300 ^oC under a nitrogen atmosphere with heating rate of 10 °C min^{-1}

3. Results and Discussion

3.1 Introduction

Figure 3.1 shows the physical condition of the samples after supercritical drying with addition of glycerol. since alginate content is 3%, the sample is much more stronger and harder to shape. The yellowish colour increase from sample AG/SPI-4 to AG/SPI-6 is due to the presence increase percentage of SPI.

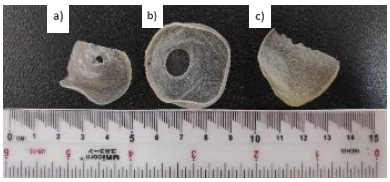

Figure 3.1: Alginate/SPI samples dried by supercritical CO_2 drying, a: AG/SPI-4, b)AG/SPI-5 and c) AG/SPI-6

Figure 3.2 shows the physical condition of the samples after supercritical drying without the addition of glycerol. It can be observed that the samples are transparent and showed low strength due to low content of 1 wt% alginate and in the absence of glycerol.

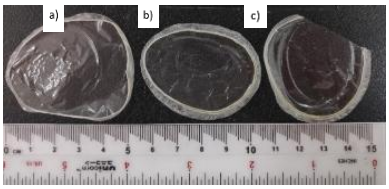

Figure 3.2: Alginate/SPI samples dried by supercritical CO_2 drying, a) AG/SPI-7, b)AG/SPI-8 and c) AG/SPI-9

3.2 Shrinking level and Tensile strength

Table 3.1: The Percentage Shrinkage of Samples Taken Before and After

No	Label	Ambient dry, (%)	Supercritical Drying, (%)
1	AG/SPI-1	60.10	42.19
2	AG/SPI-2	59.10	45.78
3	AG/SPI-3	58.22	35.11
4	AG/SPI-4	54.10	30.56
5	AG/SPI-5	53.45	30.33
6	AG/SPI-6	57.56	40.24
7	AG/SPI-7	54.14	30.11
8	AG/SPI-8	54.14	25.50
9	AG/SPI-9	50.110	23.00

Table 3.1 show the shrinking level of the composite films. The table shows 50 - 60% of shrinkage of the film after the film is subjected to ambient drying. About 23-40% of shrinkage after the supercritical drying process. The film that contain less glycerol or do not contain glycerol were brittle. This samples were unable analyzed for mechanical strength due to difficulty to cut into small pieces. The analysis was made by cutting the edge of the film which thicker than the middle region of the film. The content of the glycerol is 50% of the weight of the alginate and it shows the AG/SPI-6 a little bit sticky and flexible with good mechanical properties. In order to break the intermolecular linkage of the soy protein isolate in their original structure, plasticizer such as glycerol is used to make the protein chain able to move freely. Soy protein isolate promotes an increase of elongation of biopolymer.

3.3 Composite Films After Ambient Drying

3.3.1 Thermogravimetric analysis (TGA)

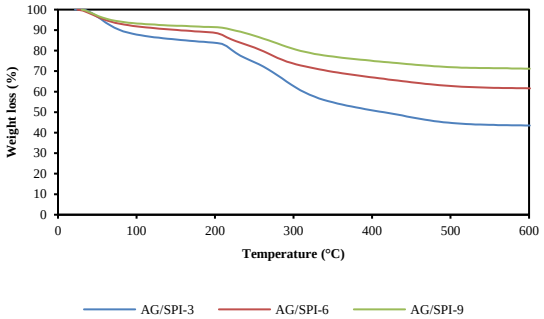

Figure 3.3: Comparison of the composite biopolymer alginate and soy protein isolate with and without addition of glycerol

During the thermal degradation in oxidative atmosphere, the SPI, SA and SPI/SA biomaterials all exhibit a three-stage decomposition. Initial weight loss 10 to 15wt% from 30 to 100 °C for alginate with addition of glycerol. Without addition of glycerol, initial weight loss about 10wt% from 20 to 100 °C. This event corresponds to water desorption. Main weight loss for the biopolymer degradations is seen at 200 to 220°C for the thermal degradation of biopolymer and it was followed by the decomposition of carbonaceous material until 500 °C. The same decomposition response of alginate based was also reported by several research [17]. Addition of glycerol cause the decomposition of composite film less stable around 203 to 209 °C. As it can be observed from the thermograms, the curve of AG/SPI biopolymer without addition of glycerol occur at 214 °C. At the final degradation stage, the 3% SPI content with addition of glycerol occurred in range 260 to 320 °C. According to [22], For pure SPI, its main weight loss is the in the temperature range from 270 to 360 °C. Based on [17], the blank alginate curve dropped at 250 °C. This

indicate that the SPI content does not have strong interaction with biopolymer backbone.

3.3.2 Fourier transform infrared spectroscopy, (FTIR) analysis

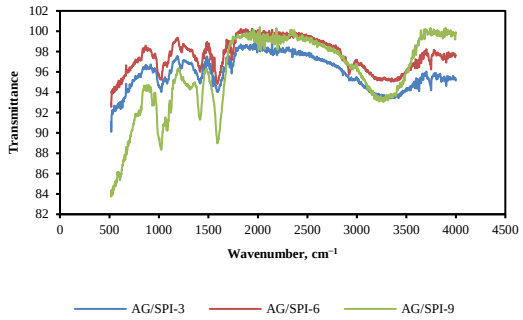

Figure 3.4: Comparison of 3% alginate with 1.5% of SPI Content with and without glycerol

FTIR is useful technique to discover the profile of the protein. The amount of water uptake is decrease with the addition of soy protein isolate about 1.5wt% due to the diffusion of water inside the network. Interaction between protein chains with alginate molecules lead to the decrease of water uptake by electrostatic and hydrophobic interactions. Regardless, SPI films do not show satisfactory mechanical properties. This is due to their innate hydrophilicity,[18].

The new absorbance rate appears at the 2958 cm−1 to 3269 cm−1 associates with N-H bod stretching. O-H stretching vibration can be seen appeared as a broad peak at 3000 cm−1 to 3600 cm−1 and 1400 cm−1 which indicate the alginate exist. A typical Amide I for the C-O stretching vibration is clearly shown at spectrum 1597 cm−1. The C-H, CO-O-CO, C-O and C=C deformation vibration can be seen in the spectral region from 809 cm−1 –1200 cm−1, there were four bands at 809 cm−1, 935 cm−1, 1022 cm−1 and 1086 cm−1. The C-H and C=C and C-O-C stretching vibration are assigned bands around 809 cm−1 and 935 cm−1. Deformation of pyranosyl rings and C-O-C vibration of glycosidic linkage in alginate can be seen at band 1022 cm−1[19].

The spectral peaks from the amide band I which are 1600 cm−1–1700 cm−1 are common the protein secondary structure. SPI which is basically protein possess heat denaturation that generally occur between 65 ºC and 70 ºC, [18]. New structural arrangements of protein can be achieved as the proteins unfold, sulfhydryl and hydrophobic groups are exposed. This followed by disulphide bonds being reformed.

3.3.3 Differential scanning calorimetry (DSC) analysis

Interconnected thermodynamic profiles can be measure by DSC analysis. The temperature and enthalpy during endothermic or exothermic processes in biopolymer materials can also be determined by using DSC analysis.

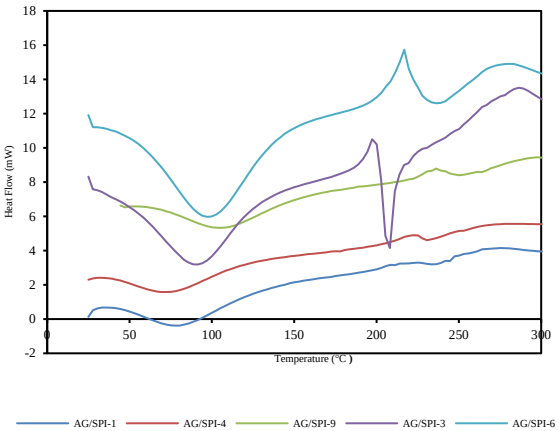

Figure 3.5: Samples AG/SPI-1, AG/SPI-4, AG/SPI-3, AG/SPI-6 and AG/SPI-9 of ambient drying

As observed in Figure 3.5, the sample with 3% alginate showing less thermal stability. The endothermic is higher than the other films for AG/SP-3 and AG/SPI-6 is most likely due to their purity and processing of the biopolymer films. Thermal properties of biopolymers depend on many factors such as their thermal treatment, natural origin, composition, purity, processing and mechanical stressing, [24]. The first peak was the endothermic events.

Figure 3.5, the endothermic band value of the polymer film that added with glycerol AG/SPI-1, AG/SPI-4, AG/SPI-3, AG/SPI-6 were 180.54 ºC, 52.55 ºC, 99.78 ºC and 91.49 ºC respectively were lower to AG/SPI-9 at 108.04 ºC. The addition of glycerol enhanced the thermal stability of the AG/SPI films based of the figure 3.5. Interaction between alginate and glycerol stabilize the SPI protein structure against eruption thus requiring a higher temperature for composite films. The Endothermic band value of the AG/SPI film is attributed to the water release from the biopolymer film in the range of 60 - 100 ºC. The hydrophobic feature present in the SPI enabled the evaporation of water with alginate and glycerol added. The following event related to the melting of the biopolymer structural chain.

This event related to the exothermic event. As observed, compared to the AG/SPI-1(216.61 ºC), AG/SP-4(205.07 ºC), AG/SPI-3 (200.11 ºC) and AG/SPI-6(216.85 ºC) presented a decrease in exothermic band value associated with thermal stability. According to [20], the exothermic band of blank alginate film is 253.82 ºC. Without the addition of glycerol, the exothermic value was 230.66 ºC. Totally, the addition of alginate and glycerol effectively

improved the thermal property for the AG/SPI composite films.

3.4 Composite Films After Supercritical Drying Process

3.4.1 Thermogravimetric analysis (TGA)

The thermal stability of the composite films alginate and soy protein was measured using TGA analysis.

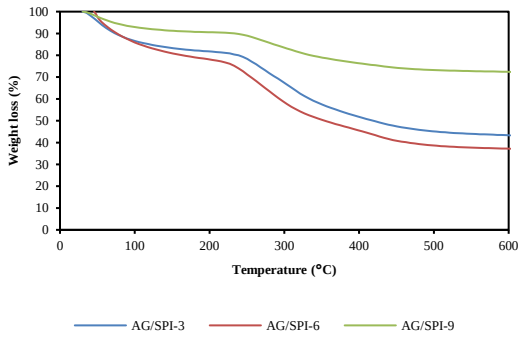

Figure 3.6: Alginate and soy protein isolate composite film with and without addition of glycerol

TGA analysis for the supercritical exhibit the same decomposition response as compare to ambient drying. three-stage decomposition of the composite can be observed in the figure. Initial weight loss 20wt% from 30 to 100 °C for alginate with addition of glycerol. Without addition of glycerol, initial weight loss about 10wt% from 20 to 100 °C. This event also corresponds to water release from the film. Glycerol content increase the degradation rate correspond to increase of the increase of glycerol.

As for the biopolymer degradation curve, it can be seen in range 230 to 250 °C for the thermal degradation of biopolymer. The decomposition of carbonaceous material occurred at 250 to 500 °C. Addition of glycerol cause the decomposition of composite film less stable around 230 to 310 °C. As it can be observed from the thermograms, the curve of AG/SPI biopolymer without addition of glycerol occur at 240 °C.

At the final degradation stage, the 3% SPI content with addition of glycerol occurred at 315 to 360 °C. According to [22], For pure SPI, its main weight loss is the in the temperature range from 270 to 360 °C. The blank alginate curve dropped at 250 °C, [17]. This indicate that the SPI content does not have strong interaction with biopolymer backbone. For all the composite films, the alginate has a typical and rapid degradation in the final stage, due to the formation of sodium oxide around 320 to 500 °C, [23].

3.4.2 Differential Scanning Calorimetry (DSC) Thermal Analysis

Glass transition temperature, Tg is point where a polymer in its molten state is cooled. This contribute to its mechanical properties such as elastic material to birttle. This is due to the change in chain mobility. However, polymer chains have high mobility at above the glass transition temperature. Heat is released to the surroundings si an exothermic process. This event is called crystallization.

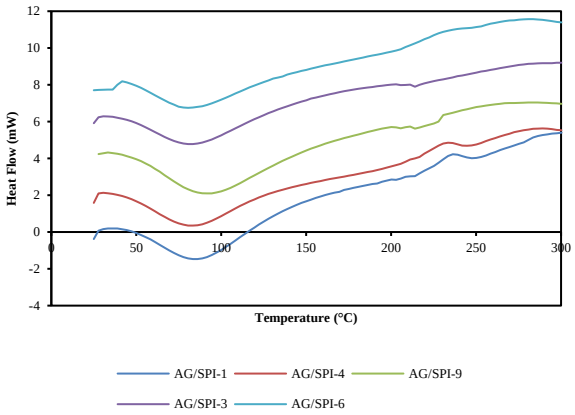

Figure 3.7: Samples AG/SPI-1, AG/SPI-4, AG/SPI-3, AG/SPI-6 and AG/SPI-9 of supercritical drying

From figure 3.7, the first peak was the endothermic events. As observed in Figure 3.7, the first peak of polymer film that added with glycerol AG/SPI-1, AG/SPI-4, AG/SPI-3, AG/SPI-6 were 80.50°C, 80.51 ºC, 83.29 ºC and 83.29 ºC respectively is lower to AG/SPI-9 which was 94.38 ºC. endothermic peak near to 100 °C attributed to the water release. Addition of the glycerol increase the thermal stability of the AG/SPI flms. This is because of the increase interchange of glycerol and alginate which result in stabilizing the protein structure against eruption.

The hydrophobic present inside the SPI cause the reduction of some weak intermolecular forces. For example the molecularforce between hydrogen bonds in water and polymer chain. This also cause the evaporation of water with alginate and glycerol added. The following event related to the melting of the biopolymer structural chain. This event related to the exothermic event. As reported, peak value of the AG/SPI flm is attributed to the hydrogen bonded networks.The exothermic band values of the AG/SPI-1(236.33 ºC), AG/SPI-4 (230.07 ºC), AG/SPI-3 (239.01 ºC) and AG/SPI-6 (241.07 ºC) presented decrease. This values associated with thermal stability. According [20], the blank alginate film exothermic values is at 253.82 °C.

[23] stated that, plasticizers cause a shift of glass transition temperature towards lower temperature. The exothermic peak indicates the oxidative degradation of alginate polymers, samer. Therefore, the addition of alginate

and glycerol effectively improved the thermal property for the AG/SPI composite flms.

3.4.3 Fourier transform infrared spectroscopy, (FTIR) analysis

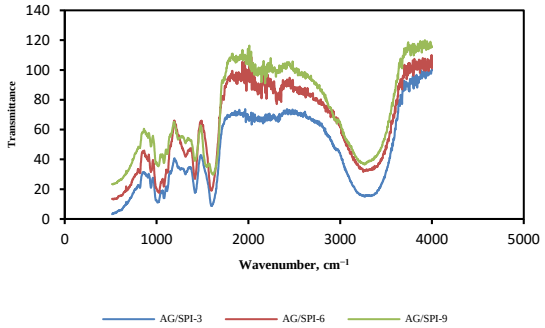

Figure 3.8: Spectrum for 3% algiante and 1.5% SPI

Notably, the peaks at 3316 and 1424 cm-1 were assigned to stretching vibrations of O-H stretching. Protein secondary structure which is amide I can be found at the peak 1610 cm−1. Deformation vibration of C-H, C=C, CO-O-CO and C-O can be found in the spectrum from 800 –1100 cm−1. There are four related bands which are 821 cm−1, 940 cm−1 , 993 cm−1 and 1086 cm−1. The C-H, C=C, CO-O-CO and C-O stretching vibration appear around 821 cm−1 , 940 cm−1 , 993 cm−1 and 1086 cm−1 bands. . A typical Amide I for the C-O stretching vibration is clearly shown at spectrum 1610 cm−1 .The presence of phenylalanine amino acid residues in the sample as protein content cause the sharp and low intensity aromatic ring breathing peak around 938 cm−1 and low intensity one at 1606 cm−1. The O-H stretching vibration can be found In spectrun range 3000-3500 shows the characteristic for alginate. This confirmed the existence of AG/SPI mixture and the remains after crosslinking.

3.6 Scanning electron microscopy (SEM)

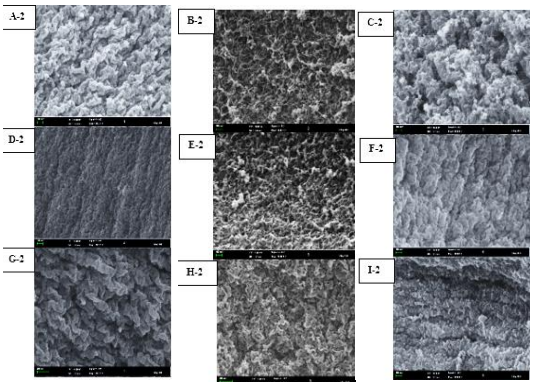

Figure 3.9: SEM images of alginate and soy protein isolate with and without addition of glycerol and their surfaces morphology for sample AG/SPI-1 (A-2), AG/SPI-2 (B-2), AG/SPI-3 (C-2), AG/SPI-4 (D-2), AG/SPI-5 (E-2), AG/SPI-6 (F-2), AG/SPI-7 (G-2), AG/SPI-8 (H-2) and AG/SPI-9 (I-2)

Addition of more alginate create the surface morphology rougher. Figure 3.9 with high magnification images A-2, B-2, C-2 ,G-2, H-2 and I-2, the samples with 1 wt.% alginate are less porous as compare to the high magnification images D-2, E-2, F-2 with 3% alginate which are much more porous on the surface of the samples. At high magnification of samples with 1 wt.% alginate, addition of SPI content lead to smoother surface.

The absence of glycerol lead to coarse surface of the bioplymer film. By referring to the surface inspections, alginate concentration had most profound effect on morphological characteristics rather than protein concentration, [19]. Alginate exhibited rough surfaces with porous and heterogeneous morphologies due to some nonfully destructured alginate particles, [20]. High porosity related to the blank alginate presents in the surface morphology interconnecting pores in the the gel matrix.

The sample exhibits smoother surface with the rise in glycerol content. Thus, the addition of soy protein led to a increase smoother surface as compared with the hydrogels of pure alginate. The AG/SPI samples showed high attraction to glycerol by having a good homogenous mixture. According to [23], excessive amount of glycerol lead to immiscibility within the microstructure of the film, [23]. Increase in the glycerol content cause increase in the interaction between the biomaterials between their functional group. According to [23] glycerol plasticizer alter the interface between the adjacent molecules in the polymer chain leading to increase in extensibility of the polymer matrix.

4. Conclusion

The use of biopolymers has been studied in recent years. The current wound dressing has disadvantages such as less flexibility, poor mechanical strength and lack of porosity. Hybrid biopolymer of alginate and soy protein isolate were successfully fabricated using solvent casting technique and dried by supercritical drying. Results demonstrated that it was possible to form a blend between alginate and SPI leading to an improvement of the mechanical properties, which can be related also to the conformation of the secondary structure of the protein. The shrinkage level of the hybrid biopolymer decreases when undergone supercritical drying about 23-40%. Increase the alginate content had increased the roughness of the biopolymer films. However, increase in the SPI content

smoothen the surface of the biopolymer whilst decrease in the alginate ratio decreased the mechanical strength of the biopolymer. This study shows that the addition of glycerol affects the thermal stability and mechanical properties of the composite films and significantly reduced the hybrid biopolymer glass transition temperature. Further studies on the antimicrobial and porosity of the hybrid biopolymer with different formulation such as addition of hydrophobic copolymers and antimicrobial polymer might enhance the biopolymer characteristics.

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