Effect of Glycerol Compositions on Protein Based Thermoplastic from Fish Waste

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Abstract. The main objective of this work was to investigate the effect of glycerol at different compositions on physical properties, morphology and water absorption of protein based thermoplastic from fish waste. Fish waste containing fish head, bones and fins were collected from local fish product processing plant before further cooking, pressing, drying and milling. Compound formulation was done using twin screw extrusion and compression moulding. Samples at different glycerol compositions (Control, 20 pph and 35 pph) were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and water absorption. The addition of glycerol showed two broad bands between 1500-1700 cm\(^{-1}\) and 3000-3500 cm\(^{-1}\) which correlates with the amount of α-helix and β-sheets structure of the protein. The fracture surfaces at control changed from uneven surfaces with large particles to smooth surfaces. Plastic deformation was observed at control and 20 pph but was absence at high glycerol content. Increasing the glycerol content was found to influence the water resistance properties of fish protein by reducing the absorption capability due to amount of α-helix and β-sheets structure.

1. Introduction

Environmental pollution caused by petroleum-based plastics is a serious problem that becoming a threat to human life, animals and atmosphere. Their increasing use for one off or short-run applications has resulted in a great concern and interest in replacing petroleum based plastics with renewable biodegradable polymer materials. Natural biopolymers such as starch, cellulose, chitin, polylactic acids, soy protein, collagen, and gelatin have been successfully transformed into thin, transparent and flexible material of biodegradable films [1, 2]. Therefore, renewable raw materials such as protein extracted from agricultural, marine, animal or microbial sources can be degraded by the environment. For example, plant protein materials such as soy protein[3], potato protein [4], wheat gluten [5], and sunflower protein [6] have been studied among researchers. Besides that, animal protein such as blood meal [7], silk protein fiber [8], and fish myofibrillar proteins[9] also has been successfully being transform into thermoplastics. The advantages of biopolymer such as sustainable sources of raw materials and they are mainly environmental. Protein consists of repeating monomer similar to synthetic polymer but may contain up to 20 amino acids monomer that form polypeptide chain. Therefore, proteins are renewable, biodegradable/edible resource with great potentials to improve the quality and stability large range of agricultural products.
However, there are some limitations because protein has lower mechanical properties as compared to synthetic materials. In previous study, waste blood has shown its ability to perform as thermoplastic polymers with high mechanical properties [10, 11]. It is important to control the material moisture content in order to ensure consistent material properties. In other research, fishmeal from Atlantic salmon has been successfully compression moulded and plasticized with glycerol [1]. Glycerol was added to either function as lubrication or increasing free volume. Moreover, glycerol has high boiling point and good stability and is regarded as one of the most efficient plasticizers for fish protein thermoplastics. Addition of plasticizer such as glycerol made soy protein thermoplastics and improved the flexibility by decreasing the tensile strength and modulus but increased its elongation [12]. Similar effect has also observed for sunflower protein that has been developed into thermo-moulded films [6]. During thermoplastic processing, protein molecule chains slide past each other and solidify to form a shape when the temperature is reduced in order to achieve flowing protein. The most common processing method for biopolymers is extruder. However, twin screw extruder offers more efficient mixing and conveying materials [10]. Some research of biopolymer uses internal mixer instead of conventional extruder [13]. In this study, fish waste from sardine is employed as raw material to evaluate whether this significant waste from fish processing industry can be converted into environmentally friendly bio plastics. This study also involves the influence of chemical additives and evaluation of a range of processing techniques such as compression moulding and extrusion.

2. Methodology

2.1 Materials

The fish waste used (Sardine) was supplied by Protigam Food Industries Sdn. Bhd (Butterworth). The fish waste is produced from fish products such as bones, heads, guts and fins. It has 46.4% of protein content (measured by Kjedahl method), 1% of fats and 3.29% of moisture. Glycerol and other chemicals including technical grade sodium dodecyl sulphate (SDS), analytical grade sodium sulphite (SS), urea, and glycerol were obtained from Sigma Aldrich.

2.2 Sample preparation

2.2.1 Fish Waste powder preparation. Fish waste was cooked at 90 °C for 20 min with a fish/water ratio of 1:1(w/w). A solid was separated by filtration and was pressed into a cake for further dried until it reach a constant weight at 90 °C and subsequently milled into a fine powder. Finally, the fish waste powder was sieved to an average particle size of 600 μm.

2.2.2 Preparation of Fish Protein Thermoplastic. Samples were prepared by dissolving urea, SDS and SS in water. The solution was heated until the temperature reached 50 °C followed by blending with fish waste powder in a high speed mixture for 5 min. Glycerol was added to the mixture and blended for another 3-4 min. The mixture was stored for at least 24 h prior to mixing process. Sample 1 was formulated without glycerol known as Control, and Sample 2 and 3 were formulated with varying amounts of glycerol as a plasticizer, and water as a secondary plasticizer, as shown below in Table 1. The samples were further processed using twin screw extruder.
Table 1. Fish protein with varying concentrations of plasticizer

| No | Formulations composition | Sample 1 (pph) | Sample 2 (pph) | Sample 3 (pph) |
|----|--------------------------|----------------|----------------|----------------|
| 1  | Fish Waste Powder        | 100            | 100            | 100            |
| 2  | Urea                     | 10             | 10             | 10             |
| 3  | SS                       | 3              | 3              | 3              |
| 4  | SDS                      | 3              | 33             | 3              |
| 5  | Water                    | 20             | 20             | 20             |
| 6  | Glycerol                 | Control        | 20             | 35             |

2.2.3 Extrusion. Sino Alloy compact co-rotating twin screw extruder-PSM30 was used to extrude fish protein mixtures. The screw diameter was 31.2 mm with a length to diameter ratio of 40. The extruder was fitted with a single 10 mm circular die and the temperature processing from zone 1 until zone 9 as shown below in Figure 1. The extrusion is performed under screw speed 100 rpm and then the extrudate was crushed.

![Figure 1. Twin-screw extruder. Screw and temperature profile [14]](image)

2.2.4 Compression- Moulding. A hydraulic press (GoTech Test Model KT-7014 A) fitted with heating elements on the top and bottom plate was used for compression moulding. A mould was employed and a Teflon sheet was placed on the bottom of the mould to prevent sticking. Processing conditions such as temperature, preheat time, compression time, cooling time and pressure were optimized. Fish protein thermoplastics was generally pre-heat about 4 min, compressed time for 5 min, cooling time about 2 min each at 120 °C and pressure 9.7 Mpa. Then, the sample was straight to analysed. The samples was performed according to the ASTM D638-02a [15].

2.3 Characterizations

2.3.1 Fourier Transform Infrared Spectroscopy (FTIR). FTIR analysis was performed using a Perkin Elmer spectrophotometer, employing 16 scans at resolution from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\). The background was scanned prior to each sample scan and automatically subtracted from the spectrum by the FTIR software.

2.3.2 Morphology. The morphology of the thermoplastic fracture surface was investigated using scanning electron microscopy (SEM) (Hitachi S3000N) at an acceleration voltage of 5 kV. A small fragment of sample was placed on aluminium stubs with double sided tape. The stubs were coated with carbon paint and placed in an ion sputter which further coated the specimen with platinum prior to SEM analysis.

2.3.3 Water Absorption. The testing was performed according to ISO 62:2008 [16]. The water absorption capacity of each sample was studied by immersing a known mass of sample into distilled water at room temperature. The sample was periodically removed from the distilled water and reweighed until constant film mass was attained. The test was performed within 3 days in triplicate.
3. Results and discussion

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of fish proteins for Control, 20 pph and 35 pph of glycerol are show in Figure 2. The appearance of a secondary peak at 3344 cm\(^{-1}\) suggests the formation of primary amine stretches. It was obvious that the peaks in the region 3100-3500 cm\(^{-1}\) shows increased in transmittance percentage and broadening which indicated the presence of NH-stretching with hydrogen bonding. The transmittance increased about 3% (20 pph) and 11% (35 pph) as compared with between peaks at control. These broadening were related to the possible extra interactions that arise between the plasticizer and the film structure. Generally, amide I (1600-1700 cm\(^{-1}\)) related to carbonyl group of proteins, amide II (1480-1575 cm\(^{-1}\)) related to vibrational groups N-H and stretching vibrations of C-N groups and Amide III (1229-1301 cm\(^{-1}\)) referred to vibration in plane C-N and N-H groups of bound amide[17]. Amides I band (highlighted in box) were found increased in absorbance and broadening about 1% and 3% when the addition of glycerol. There are 2 peaks of vibrational bands each sample in the Amide I (1600-1700 cm\(^{-1}\)) which could be associated with extended in β-sheets structure. This indicates that the interactions between polypeptide chains are located in β-structure regions and this is believed affected by temperature and concentration of molecules in forming hydrogen bonds interactions [18].

![FTIR spectra of fish protein with different composition of glycerol (Control, 20 and 35 pph)](image)

**Figure 2.** FTIR spectra of fish protein with different composition of glycerol (Control, 20 and 35 pph)

3.2 Scanning Electron Microscopy (SEM)

Figure 3 shows SEM images of fracture surface of fish protein thermoplastics with increasing composition of glycerol. It can be seen in Figure 3(a), at control shows an uneven surfaces with large particles and started to reduce when the addition of 20 pph of glycerol (Figure 3(b)). As the glycerol increased to 35 pph, an even and homogeneous surface without large particles has been observed in Figure 3(c). Although, the plastic deformation behaviour has been observed in Figure 3(a) and it shows more obvious plastic deformation when the increasing of the glycerol in Figure 3(b). This is because without plasticizer, the mixing was poor and brittle. In addition, the mixtures at control had a rough texture Figure 3(a) while the higher glycerol content had a smooth texture Figure 3(b). A material with plastic properties has good mechanical properties such as high in elongation and low in tensile strength. The addition of 35 pph of glycerol had a combination of both textures but with a higher proportion of smooth texture Figure 3(c). These results suggest that the increase of glycerol content in fish protein mixtures results in a better mixing between protein and plasticizer, which in turn, yields smaller particles and in a more smooth texture of the mixture. One possible explanation for
this was that when plasticizer content was increased there were strong protein-protein interactions disrupted and the more partial unfolding such as loss of quaternary structure and consequently, the higher swelling capacity of the fish protein network.

![Figure 3. SEM images of fish protein with different composition of glycerol (a) Control, (b) 20 pph and (c) 35 pph](image)

### 3.3 Water Absorption

Water absorption is a very important factor for many applications of bio-plastic products. The results of water absorption experiments, performed at the room temperature over 3 days are shown in Figure 4. It can be seen that the fish protein thermoplastic showed an increase of water absorption at the first stage. The absorption of all the thermoplastics was rapid during the initial 5 h followed by slower absorption rate until they reach equilibrium. This phenomenon may be due to the abundant active hydroxyl groups of glycerol which were vacant at the initial stage of absorption process. At control, the water absorption of fish protein thermoplastics has been improved to about 33 % at 72 h which is day 3. However, the addition of glycerol at 20 pph and 35 pph has reduced the water resistant of fish protein to about 28 % and 26% at day 3. It can be observed that the addition of plasticizers effectively decreased the swelling and water retaining capacity of fish protein thermoplastics. This could be explained by an increase in glycerol content caused the interactions with polypeptide chains became complex which lead to a less organised network.

![Figure 4. Water absorption of fish protein with different composition of glycerol (Control, 20 and 35 pph)](image)

### 4. Conclusion

This study showed clearly with reference to the FTIR spectra, which fish proteins shown that intermolecular β-structures were induced by the additions of plasticizers. Plasticizers and heating are the main effect to significantly increase the intensity of the β-band. It shows that the interactions between polypeptide chains are located in β-structure regions and maintained by hydrogen bonds.
Without plasticizers, the fish protein films are brittle with many plastic deformation and uneven surfaces with large particles. Hence, the introduction of plasticizers helps to overcome brittleness and produces smooth surfaces with great mixing process. Moreover, increase in plasticizer effectively decreased the swelling and water retaining of fish protein thermoplastics.

**Acknowledgement**

The authors wish to acknowledge the Short Term Research Grant and Fundamental Research Grant Scheme (FRGS) for their valuable contributions to this study.

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