UNIVERSITI PUTRA MALAYSIA

PREPARATION AND CHARACTERIZATION OF FISH-GELATIN BASED FILMS

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By

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Lastly, thank you from bottom of my heart to my dear friends and lab officers for their company and time.

Sincerely,

Nurfatin Nabila bt Tajul Arifin

ABSTRACT

The economy of freshwater fish in aquaculture production is reported to be increasing throughout the year in Malaysia and as much as 33% of the freshwater fish production has been contributed by Tilapia fish, thus, this will increase the amount of fish waste that can be a useful product. Tilapia fish can be a good resource of gelatin to replace any other gelatin that available in market nowadays, and also, HALAL issues would not be one of the major problems anymore. This research was carried out to observe the properties and characterization of fish gelatin based film with different type of plasticizers and to identify the effect of different types of plasticizers based on its mechanical and thermal properties of fish gelatin based film. As many as four different types of plasticizer with the same concentration were added into fish gelatin based film, they are, Glycerol, Sorbitol, Glycerol + Sorbitol and Diethylene Glycol. Tensile test was held to study the mechanical properties of the plasticized films and tensile strength and it elongation at break were observed. While Differential Scanning Calorimetry test was held to determine the glass temperature state of the plasticized film where glass temperature can determine the temperature at which the binding force of intermolecular structure of gelatin film to be in relaxed or free state in order to allow large-scale of molecular movements, as the movement of molecular increase, the lower the glass temperature of the plasticized films. The Thermogravimetric analysis test was held to observe the weight loss of the plasticized films in the function of temperature and Fourier Transform Infrared Spectroscopy (FTIR) test proved that the different types of plasticizer that was added in the fish gelatin based film, brings out different type of molecular bonding to be stretched and plays different functional roles. These experiments confirmed that different types of plasticizer that was added into fish gelatin based films, gives out different kind of mechanical, physical and thermal properties of the films and to concluded

that, fish waste possess the potential to be an alternative gelatin source for variety uses in many fields, also, the behavior of the plasticized films was explained in terms of molecular weight of plasticizers which demonstrated that the studied properties could be considered as functions of the number of molecules of plasticizers in the films. Finally, the main findings of this research is to prove that fish gelatin based film can be another way to substitute the bio-based films such as starch based films that have been widely used nowadays, as in results, fish gelatin based films shows a positive number based on their mechanical properties rather than starch based films, yet again both of them can be used functionally based on application that will be applied through them.

DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UPM or other institutions.

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(Nurfatin Nabila bt Tajul Arifin)

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Introduction

Chapter 1

1.1 Background study

Packaging has provide special barrier to food products and the outer environment for serving marketing and customer information purposes, it's also gives extra ability to protect the foods from contamination which its purpose to maintain the hygiene of the products and extending the shelf life of foods that likely to spoilt easily especially to foods which those potentially to oxidize and having microbial deterioration (Gomez-Guillen et al., 2009). Nowadays, petrochemical based plastic have been widely used due to their availability in abundant quantities also offers a low cost and favorable functionality characteristics such as good tensile and tear strength also it gives good barrier properties to O_2 and aroma compounds with heat stability. Petrochemical based plastics include polyolefin, polyesters, polyamides and many more, these types of plastics are obviously non-biodegradable due to its low water vapor transmission rate and therefore it brings to environmental pollution which will cause bad effect to ecological problems that may be world concern on these days (Tharanathan, 2003)

The disposal of plastics material is very hard to be done; by burning they can make toxic chemicals such as dioxins release to the air (Woodford, 2018). Collecting and recycling non-biodegradable plastics also difficult because they consist of so many different kinds and have they own recycle process, that's why people are neglecting about recycling them and buried them under soils instead. To encounter these earth problems, the interest in developing bio-degradable packaging material or edible as it called has been increasing throughout the years,

this is because bio-degradable films or plastics are environmental friendly rather than synthetic ones and non-biodegradable.

Bio-degradable plastics contain additives that causing decay more rapidly occurred with the presence of light and oxygen, moisture also heat may help too (Woodford, 2018). Bio-degradable plastics are made from all natural based products such as corn oil, orange peels, starch and also fish-scales, process of making this kind of plastics begins with extraction of material that needed to form films and mixture will then be poured in various shapes. As for this study, bio-degradable film is made of fish-scale where the gelatin from fish-scales of Tilapia has been extracted in propose methods and different types of plasticizers are added to compare mechanical strengths of the films can upholds.

Fish byproducts which are scales, skin, bones and fins account for ± 21% and can go up to ± 50% to some fish (N. Arpi, Fahrizal & M. Novita, 2017). Fish scales are simply acts as protection for their body and skins, unfortunately, it is being considered as useless for human, a waste indeed. Based on research that have been made, fish scales can be source of bioplastics, therefore this will help in reduction of waste along with fossil fuel consumption, environment conservation also a cheaper ways to make up plastics rather than having petroleum based as plastics ingredient (Alvin et al., 2009).

Gelatin that is extracted from fish scales is a protein product that produced by partial hydrolysis of collagen that contain in fish scales itself, amino acids that is linked together by amide linkages in a long molecules chain is what made up gelatin molecules (Anonymous, 2018). Collagen is the major structural of protein that can be found in animal skins and bones that is connective with their tissue, it is in soluble form which is very useful in so many various

fields such as foods, cosmetics and pharmaceutical due to its unique characteristic of biodegradability and low antigeiticity (Fehng, 2016)

1.2 Problem Statement

These days, plastic film packaging has always been concern problem rather than any type of packaging method that is available, this issues have brought up recently as about 80% of plastics packaging that usually made to pack foods is being discarded right after the first use and one-third of household waste is made up from them. This volume contributes to abundant landfills and new areas are required to dispose the waste generated by time (Anonymous, The use of plastic on food packaging: problems and solutions, 2017). Therefore, by several studies that has been made to encounter this problem, an edible thus biodegradable plastics packaging which created from fish gelatin based has become hype and innovative for industry nowadays.

Gelatin is a yellowish, odorless and tasteless that usually made by extended boiling of skin, cartilage and bones of animals, primarily made from meat industries left over and mainly sources are from pork skins, horns and cattle bones (Sugar, 2018). Halal issues has been the biggest problem throughout gelatin industry, thus, to solve this problem, gelatin that is extracted from fish waste is confidently safe and useful to any other applications such as packaging and even for consumption. Moreover, there are abundant of research that have been carried out in order to extract gelatin in many different types of fish, but as we noticed that the amount of amino acid content such as glycine have brought the main structural properties towards the outcome products, let's say a film. Hence, from this research it was clearly shown that glycine content in fish gelatin from tilapia scales was comparable to any other types of fish gelatin. Also, tilapia fish have brought as much as 33% of contribution through the freshwater fish production industry in Malaysia where it is as much as 44,099 MT of tilapia production in number per year,

fish waste such as the scales may also be the sources of gelatin, it has been estimated that more than 50% of fish tissues including the scales are rejected as they were considered as 'wastes'. They may reach 20 million tons that is equivalent to 25% of the total production of marine fishery catch every year (Caruso, 2015).

Unfortunately, few restriction on using fish based gelatin has been found, they are easily damage due to low ability to withstand strength and low durability (Panayotis, D., K., & Zotos, A., 2016). Thus, to solve this problem, different types of plasticizer were added to improve their resistance and mechanical properties also solving the current problem regarding poor properties of fish gelatin.

1.3 Objective of the study

1. To characterize the properties of fish gelatin based film with different type of plasticizers.
2. To identify the effect of plasticizers concentration on the mechanical properties of film.

Chapter 2

Literature review

2.1 Bio-degradable film packaging

Bio-degradable film were intended to be produced as a substitute ways in using the polyethylene film which can take up to 1,000 years to be degrade in landfills that emit harmful greenhouse gases thus, polluting the environment. The term of bio-degradable materials is used to describe those material which can be degraded by any enzymatic reaction that happened by living organism such as bacteria or fungus into water and carbon dioxide. Biodegradability of the polymer films will be dependent on its sources of polymer, chemical structure and also the environmental condition, generally, an increase of molecular weight of polymer will results in the decreasing of the biodegradability.

Nowadays, consumers have demanding on more eco-friendly packaging solutions as their awareness towards the environmental issues that synthetic polymer has brought up. Thus, the development of biodegradable for effective food packaging has generated considerable in recent years due to their potential to reduce the usage of conventional plastic hence, saving the earth.

2.2 Tilapia

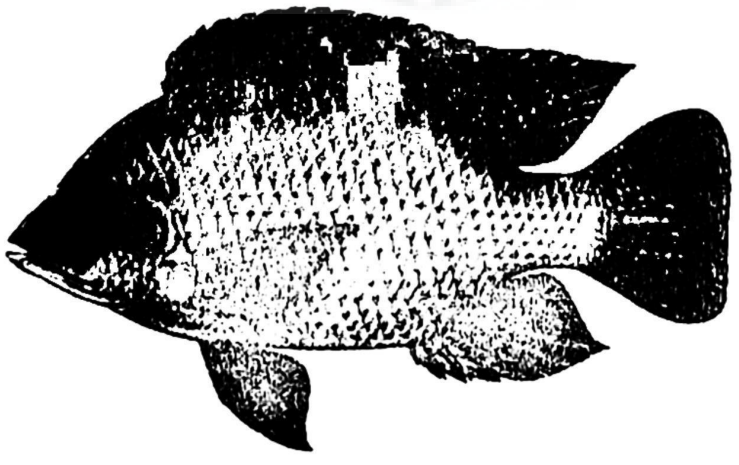


Figure 1 Tilapia fish

Tilapia or its scientific name is *Oreochromis niloticus* was first introduced to Malaysia which origin in Indonesia in the year of 1944 (Gopinath et al., 1989). Tilapia took account for almost 45% of freshwater fish population as well as production other than carps and catfish, in terms of value; tilapia also holds huge number of yields of USD 27 million. The increase rate of tilapia production causing by the high demand of tilapia fish thus contribute to the by-product of the fish.

2.3 Tilapia scales

Fish scale is a small rigid plate that outgrow on the skin of any fish, fishes that covered with these scales function to provide an effective camouflage through reflection and coloration as well as its possible hydrodynamic advantage. During fish production, as many as 50-70% of byproducts of tilapia fish including the scales were discarded from its total weight per fish (Ahmad, Fisheries Country Profile: Malaysia, 2017). Furthermore, according to Kementerian Pertanian dan Industri Asas Tani Malaysia, 2015, reported that tilapia fish have brought as much as 33% of contribution through the freshwater fish production industry in Malaysia where it is as much as 44,099 MT of tilapia production in number per year. Thus, huge number of fish scales as by product of its production has been produced throughout the year. The best part of gelatin source can be placed in fish scales or its bone, in recent studies, fish skins and its scales has been widely used as raw material in order to produce fish gelatin Tilapia scales were collected from local wet market in Jerantut, Pahang. The collected scales were immediately washed with tap water and frozen prior to its further used in next step which is extraction of its collagen thus giving gelatin as fish based film raw material.

2.4 Plasticizers

Plasticizer is a vital ingredient of the most formulation. It helps to improve the flexibility of the film and reduces the brittleness of the film, its properties of low molecular weight and non-volatile components has been the most important parameter that made it as functional to be used widely in polymer industries as an additives (Sejidov et al., 2015). Plasticizer significantly improves the film properties by reducing the glass transition temperature of the polymer (Patel et al., 2015). The selection of plasticizer will depend upon its compatibility with the polymer and also the type of solvent employed in the casting of strip. The flow of polymer will get better with the use of plasticizer and enhances the strength of the polymer.

For the past few decades, plasticizer has been produce worldwide as many as 5 million tons per year that were brought up to as much as 60 types of polymer and more than 30 groups of products produced by application of plasticizers. The application of plasticizer in order to modify the characteristics of polymer have started in 1800s which is this application is no longer a new practice ever since it was started (Bialecka-Florjanczyk et al., 2017). Nowadays, natural-based plasticizers that are characterized by low toxicity and low permeability have caught so much interest to the polymer developer, this group of natural-based plasticizers include epoxidized triglyceride vegetable oils mainly from soybean oil also not to be missed, fatty acid esters (Balkose et al., 1999). Moreover, the increasing interest of natural-based plasticizers that made from renewable and bio-degradable resources development has been widen among researchers and industry is due to their potential in order to reduce the usage of conventional plastic and most importantly, the plasticizer used also have to be in bio-based material.

2.5 Types of Extraction

Gelatin from fish scales can be extracted in some different ways based on the research practices and its purposes, the breakage of cross-links and non-covalent bonds of collagen is basically explained how do gelatin is extracted. Method involves could be done by direct thermal treatment, use of acidic or alkaline and lastly, enzymatic pretreatments (Johns, P. & A. Courts,, 1977). As for this research and mostly used method which is acidic and alkaline pre-treatment have brought an advantaged over the other method such as direct thermal treatment which is carried out under a high temperature and production of gel inferior quality is likely to be happened. However, different types of extraction will gives out different yields, chemical composition and also characteristics of extracted collagen from one another.

In recent works, there are abundant types of gelatin extraction from different types of fish species that has been carried out, they are, Alaskan Pollock skin by Zhou & Regenstein, 2004, yellow-fin tuna (Duan et al., 2009), Nile perch skin and bone (Nagai et al., 2000) and many more in count. Type A gelatin or gelatin that extracted from acid-treated collagen has been widely reported type of gelatin that is derived from fish by products material, it is confirmed that acidic treatment is the most suitable method for fish byproducts due to its less covalently cross-linked collagen (Karim, A.,A., & Bhat, R., 2009). Furthermore, precious report also mentioned that alkaline treatment which followed after acidic neutralization provide a neutral or weak acid extraction medium that makes production of gelatin yield in higher value.

2.6 Gelatin Extraction Method

Gelatin from tilapia fish scales were extracted according to (Limpisophon et al., 2009) method with some modifications has been applied according to the suitable extraction condition. Firstly, fish scales were washed with tap water for three times in the scales of 1:10 where

100gram of fish scales needs 1000ml of water, this process is important to remove bloods and dirt residue that embedded on the fish scales. Next, fish scales will undergo alkaline treatment where they are soaked in 0.05M of NaOH and 25% of methanol solutions with scale to solution ratio of 1:4. Fish scales are left overnight in the freezer at 4°C in the alkaline solution to remove lipids and non-collagen protein in the fish scales. Alkaline treated scales again will be washed with tap water for three times to obtain a neutral pH. Then, demineralization of tilapia scales takes place by soaking the scales in 0.05M of HCL solution for two hours at room temperature. Fish scales were washed again by using tap water to obtain neutral pH of the scales before proceeding to next step. After pre-treatment, the swollen scales were soaked in distilled water at 80°C for one hour this steps contribute to the extraction of gelatin process, and the extract gelatins obtained will be centrifuge for 30 minutes at 15,000×g. Lastly, the supernatant were freeze dried in the freeze dryer for three to four days. Gelatin form was stored at -18°C in the freezer until the next use.

2.6.1 Amino Acid Content in Collagen Extracted

It has been reported that tilapia fish's collagen contained of high amount of glycine and proline group yet deficient in essential amino acid such as lysine and tryptophan based on research studies. The three polypeptide chain or alpha-chain that made up collagen monomer called as tropocollagen and cylindrical protein is having molecular weight that reached 100,000 daltons for each (Wong, 1994). These three chains are linked together through cross intermolecular bonds giving rigidity to collagen structure and poor solubility properties. The amino acids sequence of collagen's polypeptide chain is characterized by the repition of Gly-X-Y, where normally, X position is holds by proline and Y position is occupied by hydroxiproline. Glycine content in the collagen's polypeptide chain becomes the helical center of the molecules,

this is due to glycine has only obtain of hydrogen side chain. The molecular structure is strengthening by the exhibit of Proline and Hydroxyproline in the structure (Nagai et al., 2008).

2.7 Fish gelatin

Fish processing industry has been generated as much as 50% to 80% of solid wastes from the original raw material (Joseph et al., 2007), these wastes or by-product were known to contain high amount of protein that makes them suitable to be as a raw material for another useful purposed used. According to Fisheries Country Profile (Ahmad, 2017), Malaysia's aquaculture to be specific freshwater fish production for human consumption have achieved production as much as 250,000 Metric Ton (MT) in the year of 2015, this huge amount will produced abundant of fish waste as per discussed before. Nowadays, the easiest way to understand the concept of by-product is all the raw material, inedible or edible that left during the production of main products.

A considerable attention by users toward fish by-products as an alternative source of collagen or gelatin have going through since they were introduced in recent years. In recent report indicate that as many as 326,000 tons of pig skin gelatin has took over 46% of annual world output of gelatin (Karim et al., 2009), since pig-skin have brought a big problem in HALAL industry and of course to Muslim users, fish by-product gelatin based as an alternative ways has been catching the heart of users. Furthermore, the news about the outbreaks of bovine spongiform encephalopathy have become a burdensome issues for manufacturer and industry, uses of bovine derived gelatin took 29.4% from annual world output gelatin, it's a lot of amount that needed to be particularly processed to avoid any harmful product to the consumer. As an alternative, fish scales, skins, and bones are a potential source of gelatin by fish waste which are,

they usually being discarded due to filleting also they were generating in large amount produced by fish processing industry (Harvinder, 2006)

2.8 Gelatin Film

Gelatins were characterized by the absence of an appreciable odour, a natural water soluble protein and random arrangement of polypeptide chains contain in an aqueous solution. These criteria of gelatin in making a based film are important in order to follow the green technologies in nowadays demand to save the earth. Some research studies has been done by many to investigate the overall effect of the addition of different substances such as plasticizers or additives with antimicrobial also antioxidant properties in gelatin based film, this research purpose is nonetheless to improve the functional properties of gelatin based film (Marina et al., 2016).

2.9 Film preparation

Gelatin powder was dissolved with distilled water and stirred at 60°C for 30 minutes to ensure final protein concentration of 2% (w/v) was obtained. Different types of plasticizers were added into different beaker consist of this solution they were glycerol, sorbitol and tri-ethylamine. Different types of plasticizer were added at different concentration of 20%, 30% and 40% (w/w) of gelatin. Gelatin and plasticizer solution will undergo sonication process to remove bubble in order to obtain a perfect form and quality of film making. Then film forming solution (FFS) was poured onto a rimmed silicone resin plate (50 × 50 mm) placing on a level surface and dried in an environmental temperature of room for two to three days. Then the resulting films were manually peeled off.

2.10 Analysis of Fish Gelatin based Film.

2.10.1 Mechanical Test

Mechanical testing of polymers is a crucial part of the development and the production process of the product, it allows the developers of plastic films or any other packaging to understand their product much better and introduce stronger quality control. Also, mechanical test of packaging product can ensure that the material complies with industrial specifications and its desired function. This test will include tensile, flexural, shear and compressive properties to be observed. Based on previous study (Farhan, A., & Norziah, M., N., 2016), they have been reported that control film which is the one that is not plasticized showed the lowest number in tensile strength and Elongation at Break (EAB) compared to films that is plasticized with glycerol and sorbitol. This study can be contributed to the strong polymer-polymer interaction formed via hydrogen bonding between the molecular chains of the raw material its self. In present study, the effect of plasticizer according to its mechanical properties of the films was measured to develop customized packaging material for further development.

2.10.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis is an analytical technique used to identify organic, polymeric and also inorganic material in some cases, it uses infrared light to scan sample material and observe its chemical properties. The mechanism of FTIR is that it will send an infrared radiation of about 10,000 to 100 cm^{-1} through a sample with some radiation absorbed and some will passed through them. The absorbed radiation through the sample will be converted into a rotational or vibrational energy by the sample molecules and resulting a signal at FTIR detector presents as a spectrum. These signals will represent a molecular fingerprint of the sample, each molecule or

chemical structure contain in sample material will produce a unique structure of fingerprint, thus making FTIR analysis is a great tool to identify chemicals obtain in the sample.

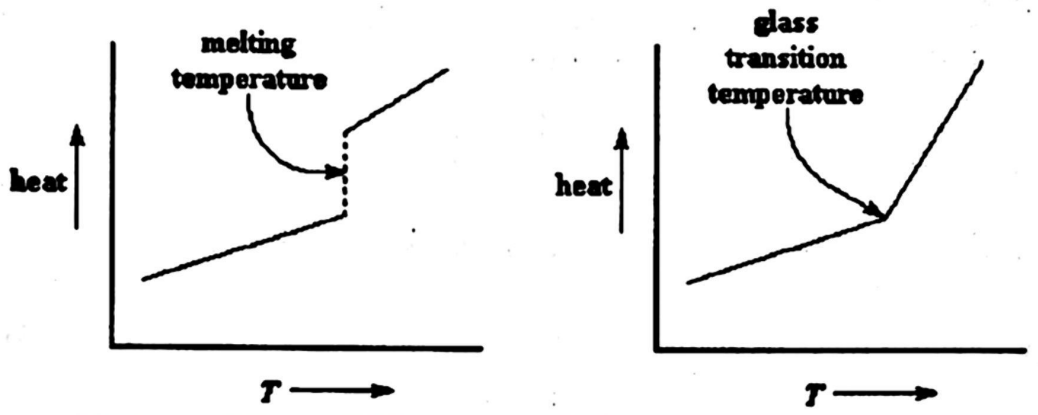
2.10.3 Differential Scanning Calorimetry (DSC)

DSC is a technique used to study changing behavior towards a polymer when they are being heated or what is called as thermal transitions or glass transition of a polymer. There are two pans in DSC instrument, one is reference and one is sample pan where polymer sample were put while the reference pan is left empty. Both pans were sits on top of a heater, DSC system will heat both sample in the same rate throughout the test is held. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle physical changes, such as glass transitions.

2.10.4 Thermogravimetric Analysis (TGA)

TGA test was held to observes and measured the amount of weight change of a material as either the function of increasing in temperature, or being isothermally function with time, in atmosphere of nitrogen, helium or any gases as low as 30mTorr. This technique can characterize the materials that exhibit a weight loss or gaining due to sorption or desorption of volatiles and reduction. TGA consist of sample pan that is supported by precision balance, this pan will reside in a furnace and heated or cooled during test was held according to the findings. Mass of the sample will be monitored through the experiment, an inert or reactive gas will act as sample purge gas that controls the sample environment and flows over the sample and finally exits through and exhaust (PerkinElmer, Inc.)

2.10.5 Glass Transition (T_g)



A heat vs. temperature plot for an crystalline polymer, on the left; and a amorphous polymer on the right.

Figure 2: Glass Transition Temperature for Crystalline and Amorphous Polymer

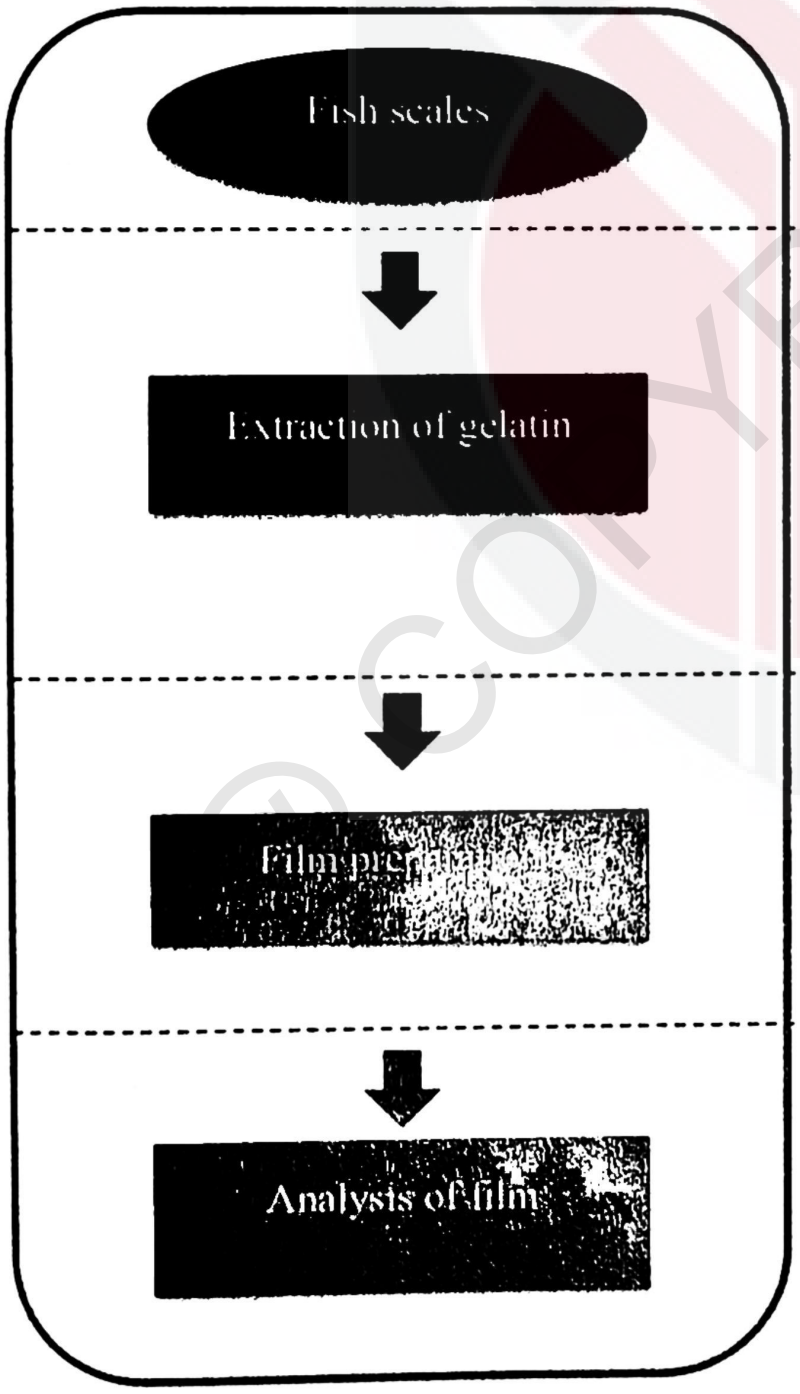
Glass transition temperature can be defined as a temperature range where a thermosetting polymer or a crystalline type of polymer changes from a hard, rigid or glassy state to a more pliable or rubbery state as shown in figure 1 above.

Chapter 3

Methodology

3.1 Introduction

Process of making a gelatin based film is start with the extraction of gelatin from Tilapia fish scale that will be discuss further soon, fish gelatin in powder form will be added into distilled water and plasticizer according to the sufficient amount that has been calculated according to previous research work and finally film casting will be done, data and graph obtained from mechanical test of the film were discussed and plotted in graph for an easier view.



3.2 Extraction of Fish Gelatin

As stated in 2.3 which is extraction of gelatin in Literature Review part, method of extraction is obtained from (Limpisophon et al., 2009) with some modification that suited our aim of end product. Firstly, 50g fish scales are soaked with tap water to washed dirt that might be embedded on fish scales surfaces, this process took three times of washing scales through running tap water. Alkaline treatment took place after scales were washed thoroughly with tap water, alkaline solution that contains 0.05M NaOH and 25% of methanol with ratio of 1:4 with scales. It took an overnight process of removing lipid also non-collagen protein in chiller that set up to be in 10 degree Celsius in temperature.

Next, after alkaline treatment have done, fish scales again will be washed thoroughly with tap water to wash off alkaline residue and returning the fish scales to neutral pH. For demineralization of scales, it occurs when acid treatment was done towards fish scales where it will be soaked in 0.05M of HCL solution for 2 hours. After 2 hours, swollen fish scales were soaked in distilled water at 80 degree Celsius for 1 hour and after that the extract of fish scales were centrifuged at 15000xg for 30 minutes. Finally supernatant was freeze dried and gelatin as shown in Figure 3 below was stored in freezer at -18 degree Celsius until further uses.

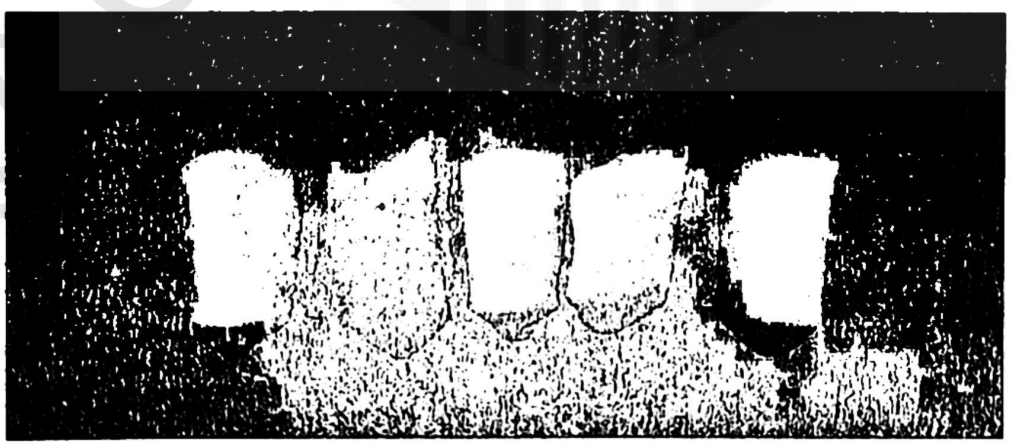


Figure 3: Tilapia Fish Scales Gelatin

3.3 Film Preparation

Film preparation is according to Lowry Method (Lowry et al., 1951) firstly, distilled water as much as 98mL were heated and stirred at 60 degree Celsius. 2 gram of gelation which is fish scales gelatin that has been extracted also various types of plasticizer were added into distilled water and stirred for 30 minutes at 60 degree Celsius then the solution were let cooled before next procedure is taken. Before film casting is done, we need to sonicate the solution first to remove any tiny bubble in order to prevent any damage to the film produced. Finally Then film forming solution (FFS) was poured onto a rimmed silicone resin plate (50 × 50 mm) placing on a level surface and dried in an environmental temperature of room at 28 degree Celsius for 24 h or more until it dried as shown below in Figure 3. Then the resulting films were manually peeled off.

Figure 4: Film Casting

3.4 Calculation of Extracted Yield Gelatin

Yield of fish gelatin was obtained by measuring the weight of dried gelatin extracted from the weight of fish scales used. Equation 3.1 below stated the gelatin yield estimation used in this experiment obtained from (Muhammad et al., 2016).

$$Gelatin\ Yield\ (\%) = \frac{Dry\ weight\ of\ gelatin\ (g)}{Dry\ weight\ of\ fish\ waste\ used\ (g)} \times 100\%$$

3.5 Mechanical Properties – Tensile Strength and Elongation at Break

The film thickness were measured by using a micrometer (Thickness Gauge; Ozaki MFG Co., Tokyo, Japan) to the nearest 0.001 mm at five random spots on the films and texture analyzer (TMS-PRO; Food Technology Co., USA) with 100 N load of cell were used to measured tensile strength (TS) and elongation at break (EAB). Three to four rectangular shape of strips with width 15mm and length 60mm were cut from each gelatin film to measure the mechanical properties of it. Mechanical crosshead speed at 0.5 mm/sec were set to start the analysis and some of the setting were set up according to the analysis desired, height and force were calibrate too before the analysis began as shown below in Figure 5. From the graph of tensile obtained, TS in MPA and EAB (%) can be calculated by dividing the maximum load (N) necessary to pull sample apart by the cross-sectional area (m^2) and dividing the film elongation at the moment of rupture by the initial grip length of sample strips then multiplied them by 100 respectively. Each test took five samples from each type of plasticizers used.

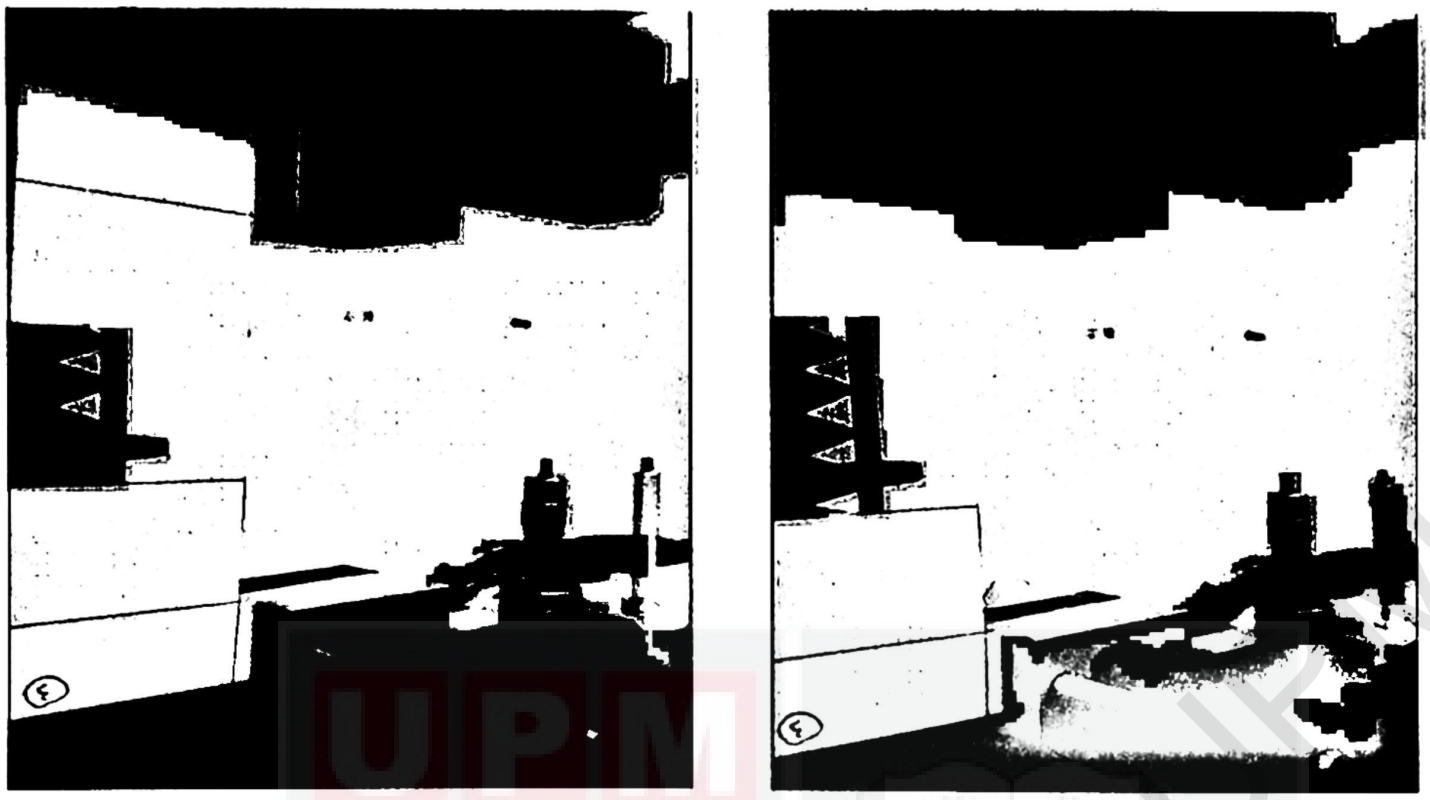


Figure 5: Mechanical Test on Fish Gelatin Plasticized Films

3.6 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (Universal V3-9A TA Instrument, New Castle, PA, USA) equipment was used to determine the glass transition temperature of different type of plasticized films. The calibration of the equipment was conducted using indium as standard. For differential scanning calorimetry (DSC) analyses, 5 mg of film samples were weighted and placed in an aluminum sample pan which was immediately sealed. An empty sample pan was used as reference. Film samples were heated from 30 to 195 °C at a rate of 10 °C/min. Nitrogen gas was used to flush the DSC cell at a flow rate of 50 mL/min to maintain an inert environment. The transition temperatures were determined from the thermogram results.

3.7 Thermogravimetric Analysis (TGA)

Thermo-gravimetric tests were performed using Mettler Toledo thermal analyzer (TA Instruments, New Castle, PA, USA).The tests were carried out at temperature range from 25 to 800 °C with a heating rate of 10 °C/min under nitrogen gas. Film sample of 5–15 mg was placed

in the sample pan and heated. The weight loss as function of temperature was depicted as thermal-gravimetric analysis (TGA) curve.

3.8 Fourier-transform infrared spectroscopy (FTIR)

The sample was sent to Material Characterization Laboratory (MCL) Faculty of Engineering University Putra Malaysia (UPM). All spectra were obtained and examined using a FTIR (Omnic spectra) spectrophotometer (Perkin Elmer, USA). A measurement technique whereby spectra are collected based on measurements of the temporal coherence of a radiative source. A total of 0.5 g film was fixed onto the crystal and knob of the Nicole i50 and placed on the FTIR apparatus. At an interval of 1.0 cm-1, each sample was subjected to 32 scans from 4000 to 400 cm^{-1} at a resolution of 4.0 cm^{-1}. s.

Chapter 4

Results and Discussion

4.1 Extraction of gelatin

Gelatin was extracted from Tilapia's fish scale according to procedure of Limpisophon et al. (2009) and Weng and Wu (2015) with slight modification. Scales of Tilapia fish was obtained from a local wet market in Jerantut, Pahang and for the storing purposes; fish scales obtained were stored frozen in refrigerator at -20 degree Celsius until further used. Conversion of collagen into soluble gelatin can be achieved by heating the collagen in either acid or alkali solution. The length of the polypeptides chain and the functional properties of gelatin will affected by the extraction process, this will also depending on the suitable processing parameters, such as temperature and its time.

Gomez et al., 2002 reported that, a procedure for extracting gelatin with high gelling capacity from fish skin is usually based on a mild acid pre-treatment for collagen swelling then, followed by the extraction of gelatin in water at moderate temperature which is 45 degree Celsius. Process would takes about 24 hours to extract the gelatin due to the acid likely to change in the crosslinks were founded in the fish skin collagen. Mild acid treatment is sufficient enough to produce swelling and disrupt the non-covalent intra- and intermolecular bonds (Montero et al., 2002). Following thermal treatment above 40 degree Celsius which is above its helix-to-coil transition temperature for fish gelatin will destroys the hydrogen bonding and discard a number of covalent bonds, thus, resulting in the conversion of soluble gelatin. In addition, for 50 grams of fish scales, as much as 5 grams of gelatin yield was produced, calculation has based on equation section 3.4 above.

4.1.1 Amino Acid Composition in Gelatin Extracted

There are differences between mammalian and aquatic living in term of their amino acid composition as it will affects the molecular structure of its own collagen. The collagen's structure and composition in solution will then affects its physical properties, it is one of the most common proteins and due to its fibrous nature, collagen will provide structural rigidity to the connective tissues such as scales and skins (David, R., V., Jaime, L., & Dalia, L., 2018).

Collagen triple helix consist of repeating sequences of X-Y-Gly in which X is Proline, Y is Hydroxyproline and Gly will represent Glycine, this statement is supported with a result of analysis that has been made. From the results obtained in amino acid analysis which was done in University Kebangsaan Malaysia (UKM) laboratory, found out that the amount of glycine had given the highest value with the average of 20.792g/100g amino acid, followed by hydroxyproline which holds as much as 14.380g/100g of amino acid and Proline value contain in 100gram amino acid was 10.466g. Each of them has their own characteristics that make it functional to the structure of the collagen.

The ability of the pyrolidine rings which consists of proline and hydroxyproline to open the geometry of the peptide chain in the regions which they happened to locate acts as important factor for collagen's molecular structure (Harrington, 1958). Hydroxyproline holds an important role in collagen stability mainly because of it is crucial to the formation of intramolecular hydrogen bonds, presences of this type of amino acid have promote the initiation of hydrogen bonding between hydroxyproline residues that is belong to the X and Y position in the adjacent chains (Aiah et al., 2015), they was initially been proposed that the collagen's stabilization was due to the hydrogen bonding network linking the prolyl hydroxyl groups with the main chain of carbonyl group that was formed by water molecules. The ladder of hydrogen that is formed in

crystal structure is important to hold the triple helix together, this case will enable the detailed study of the structure and stability of collagen triple helices (Mathew D., S., & Ronald T., R., 2009) thus, stability will affect the physical properties of the collagen. Higher thermal stability also makes the collagen thus the gelatin suitable for many food and industrial applications.

Due to glycine simplest structure and has no side chain, it may fit in many places where other type of amino acids can do, hence, it will provide the structural compactness to the collagen. Glycine also plays an important role in fibrous structural proteins where in collagen, glycerin is needed at every third position due to the gathering of the triple helix puts this type of amino acids residue at the axis of the helix, which where there is no available space for a larger side group compared to glycine with single hydrogen atom. High glycine content in collagen is important with respect to hydroxypoline and proline function to stabilize the collagen helix as glycine will allows the very close association of the collagen fibers within the molecules, facilitating the hydrogen bonding formation also the formation of intermolecular cross-links, as to be proven this statement the comparison for amino acids content between tilapia fish scales and grass carp fish scales collagen have been attached in Table 1 below according to (Mahadevappa K., 2018). Reason of comparison between tilapia scales collagen with grass carp fish scales collagen is to prove that the amount of Glycine, Hydroxyproline and Proline in Tilapia fish scales have better amount rather than another fish, thus, the stability of tilapia fish scales collagen were much more suitable to be produced to biodegradable film that can withstand some application required.

Table 1: Comparison of Amino Acids Content in Tilapia Fish Scales and Grass Carp Fish Scales

(Residues of amino acid per 100 total residues)

Amino Acid (%) / Species	Grass Carp Fish	Tilapia	Commercial Gelatin
[redacted]			
Hydroxyproline	0.82	14.38	6.49
[redacted]			

4.2 Preparation of Film Casting

The fish gelatin based film were prepared by conventional solution-casting technique with some addition of different types of plasticizers which are, Glycerol (G), Sorbitol (S), combination of Glycerol-Sorbitol for 80:20 which is 80% amount of glycerol and 20% amount of sorbitol respectively and also Di-ethylene Glycol (DG) to observed the effect and behavior of each individual plasticizer in the developed films. In order to cast out an edible film, a natural polymer such as fish gelatin has to be added to contribute the forming of a cohesive and continuous matrix structure (Bourtoom, 2008). Cohesion forces include covalent, ionic and hydrogen bonds were responsible for the functional properties of an edible film, while the cohesive strength of the films will dependent on the concentrations and type of plasticizer use (Homeiraa, 2016).

Natural polymer which is the fish gelatin will be dissolved in solvent which was water for 98ml, in this process, plasticizers, (G), (S), (GS) and (DG) also will be added, all of them have

been added at the concentration of 30% in value which brought to 0.6g of plasticizer in amount, and in order to facilitate the dispersion of gelatin into the solution, heating the solutions will help it out. Finally, this will allows the productions of smoother and consistent film, solution is then casted and dried at the most favorable temperature which is 28 degree Celsius and 56% to 57% relative humidity for specific time mostly for two to three days. These will results of the production of a free-standing film as shown in Figure 6 below.

Figure 6: Free standing film

4.2.1 Addition of Plasticizers

As mentioned in section 2.5 above, plasticizer is a material particularly to be gaseous or liquid, which is compatible with the polymer thus absorbing into the space around the polymer molecular chain. Due to its compatibility of polymer, they are able to permeate into the free volume among the polymer chains, this permeation or interaction between polymer and

plasticizer has results in few things. Firstly, it will reduces the effect of secondary bonding forces which tend to keep the polymer molecules to be stick together, next, plasticizer and polymer interaction will also increases the intermolecular distance which results in swelling of the structure also the increasing of free volume (Rich, 2004). Finally, due to the increasing of free volume and the decreasing of secondary bonding forces, plasticizer will acts so much like lubricant which will enhances the mobility of polymer molecular chain, thus resulting in more easily to move and slip past to each another in respond to an applied load where in easy words, higher elongation at break.

Introduction of plasticizer into polymer molecular chain will reduce the glass transition temperature (T_g) of the material which they will allow the polymer to remain its ductility at lower temperatures thus, this enhance the material's low temperature toughness and impact resistance. Plasticizing system can be represent of two or more component, when several plasticizers are present in the molecular chain system, strong plasticizer-plasticizer or plasticizer-water interactions can be observes. These interactions can be functional to improve the properties of biodegradable materials, include tensile, elongation at break also the other more.

For this research, plasticizers such as Glycerol (G), Sorbitol (S) and Diethylene Glycol (DG) has been used, as G-plasticizer and S-plasticizer were added in the plastic film solution, the tensile strength and elongation at break were showing an excellent number rather than DI-plasticizer, this is due the similarity of Sorbitol and Glycerol in its molecular structure unit which tends to interact with the gelatin polymer chain easily (Zhong et al., 2012). For Diethylene glycol, less number of hydrogen bonding in its molecular structure units makes it less functional in order to obtain the optimal number of tensile, elongation at break and other properties for biodegradable film.

4.3 Effect of Plasticizer on Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was held to identify the polymeric structure of fish-gelatin based film, the structures of plasticized film were analyzed by using FTIR method, where different types of amide where found to be revealed in the plasticized fish gelatin based film. The presents of Amide type A, I, II and III for control film which ones that have no plasticizer added in the films and different type of plasticized films were listed as in Table 1 shown below.

Table 2: Amide Band Wavenumber for Different Types of Plasticized Fish Gelatin Based Films

Amide Bands	Wavenumber (cm^{-1})				
	Control	Glycerol	Sorbitol	Glycerol+Sorbitol	Diethylene Glycol
A	3317.25	3319.29	3312.93	3317.83	3318.37
I	1751.16	1748.89	1740.68	1741.20	1740.54
II	1643.37	1647.78	1646.53	1647.92	1649.03
III	1241.31	1240.06	1237.69	1240.34	1237.30

Data for Amide Bands range were based on the Table 4 attached in appendices that is reported by (Sigma-Aldrich, 2019). Firstly, the Amide A band in the plasticized films were mainly indicate to the present of N-H stretching vibrations which happened to be in the range of 3310-3350 cm^{-1}. As shown in Table 1 above, all different types of plasticized films have the present of N-H bending stretching and this condition contribute to the present of amides group. Next, Amide band I were mainly associated to the stretching vibrations of carbonyls group along the polypeptide backbone of the plasticized films, and this can be indicate to the present of C=O

stretching in the plasticized films, based on the results obtain in Table 1, G-plasticized films happened to do not having this kind of molecular structure, this condition prove by its stress vs strain in discussion 4. 6 where sorbitol plasticized film which obtain C=O group reaction giving the highest peak of strain and stress value before it was deform.

Moreover, Amide band II can be determined by the present of N-H bending vibrations in the range of 1650-1580 cm^{-1}, from the data tabled above, shown that N-H bending vibration were presents at all different types of plasticized films, N-H bending vibration can be contributed also to the present of amine group in the plasticized films. Finally, Amide band III was indicated to the present of C-H bending in the plasticized films.

4.4 Effect of Plasticizer on Glass Transition Temperature (T_g)

Glass transition temperature (T_g) is the most important thermophysical transitions for gelatin based films just like any other type of films, to be specific, T_g is the temperature at which the binding force of intermolecular structure of gelatin are relaxed or free to allow large-scale of molecular movements. Thus, the T_g of Unplasticized and different plasticized fish gelatin based films were obtained by Differential Scanning Calorimetry (DSC) test and results were as shown below in Table 1. The results from previous research from (Muhammed et al., 2015) shows that unplasticized fish gelatin film has a T_g of 145.19 degree Celsius, as plasticizer were introduced to the films, it has reduced the T_g according to the plasticizer types. Obtained results were proves to several finding that reported the decrease of T_g as plasticizers are incorporated into gelatin based films, eventually, the polymer matrix becoming less dense and the polymer mobility chain is increases (Sanyang et al., 2015).

GS-plasticized films were showing a lot reduction of T_g with only 60.38 degree Celsius to compared with others, this condition can be explained to the high hygroscopic characteristic of glycerol since the amount of glycerol contribute to 80% of the plasticizers amount in that film, which glycerol that tends to retain water easily into films matrix. Addition of glycerol into fish gelatin based films will generates more hydrophilic hydroxyl groups as active sites which then will be occupied by water molecule. Thus, this condition will reflect to higher moisture content of fish gelatin based films (Sanyang et al., 2015), moisture content will affect their T_g as water is known to have plasticizing effect and also could be considered as a polymer inter-chain molecular enhancer so finally, the free volume of films will be increase and then lowered the T_g values (Mali et al., 2006). Plus, the present of sorbitol in GS-plasticized films have brought even more interactions to be present during the reactions towards plasticizer-gelatin and plasticizer-plasticizer bonding, thus, reducing the T_g easily. This amount of the lowest T_g obtain in GS-plasticized films have also contribute to the highest number of its elongation at break because of the freely molecules movement that makes it harder to break just like in discussion 4.6.2 below.

As seen in Table 1 below, S-plasticized type of films exhibits the highest amount of T_g values at 79.98 degree Celsius, sorbitol as known it have a low moisture content rather than glycerol (Rajan et al., 2006), it will forms strong interaction with the intermolecular chain of fish gelatin films due to its high molecular structure that similar with glucose units, hence, reducing the possibility of plasticizers-water interactions to occur. Strong interaction of molecular structure between sorbitol and gelatin molecules have also brings the effect to its mechanical properties where the ductility of the films will be higher, thus, reflect back to its bonding mechanisms, the higher the bond attraction the higher the glass transition temperature.

From table 3 and mentioned above, GS- plasticized film has offered the lowest glass transition temperature value for 60.38 degree Celsius, this amount of number makes GS-plasticized film to be applicable as boil-in the bag food packaging imitating nylon fibers characteristic that they varies at 50 to 80 degree Celsius (Turner, 2019) for glass transition,. This application is suitable for GS-plasticized film as food packaging as they offered toughness and low-gas permeability as well as coupled with its temperature resistance.

Table 3 Effect of Plasticizer type on Glass Transition Temperature

No.	Type of plasticizer used	Glass Transition Temperature (°C)
1.	Glycerol (30%)	61.64
2.	Sorbitol (30%)	79.98
3.	Glycerol + Sorbitol (80:20)	60.38
4.	Diethylene Glycol (30%)	66.76

4.5 Effect of Plasticizer on Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis were meant to measures the amount of weight change of a material usually in the functions of temperature, as seen in Table 2 shown below, weight losses upon the heating temperature occurred in three main steps. The initial stage of thermal degradation of films occurred at temperature less than 150 degree Celsius, weight losses at this range can be associated due to the evaporation or dehydration of the loosely bound water and low molecular weight compounds in the films (Dang et al., 2015). DI-plasticized films have shown the highest number of weight losses in this range of temperature due to its higher moisture content than other type of plasticized films.

Next, the second stage of thermal degradation of films occurred at temperature ranges of below than 300 degree Celsius corresponds to the evaporation of plasticizer compound in the films together with absorption of water molecules. This time, sorbitol conquered the highest amount of weight losses in thermal degradation as much as 0.5831mg, Rozyanti, M., (2016) reported that the degradation temperature of sorbitol-rich phase is located between 170°C-250°C where at this temperature, sorbitol begins to decompose more rapidly.

Further heating beyond 300 degree Celsius indicated as the highest value of thermal degradation rate which will reflect by the rapid change of weight reduction in all kind of plasticized films, this stage is corresponds with the elimination of hydrogen groups, decomposition and depolymerization of the gelatin carbon chains (Nascimento et al., 2012). It can be observed that G-plasticized films has the strongest resistance towards temperature, this can be explained by the glycerol-gelatin molecular interaction have reduced the strength of intermolecular bonds between gelatin molecules.

Table 4: Weight losses upon in the function of temperature

No.	Temperature range (°C)	Weight Losses (mg)			
1.	< 150	0.0590	0.1100	0.1107	0.1338
2.	< 300	0.1826	0.5300	0.5831	0.3756
3.	< 500	0.4377	0.4376	0.4742	0.6155

4.6 Mechanical Properties of Plasticized Films

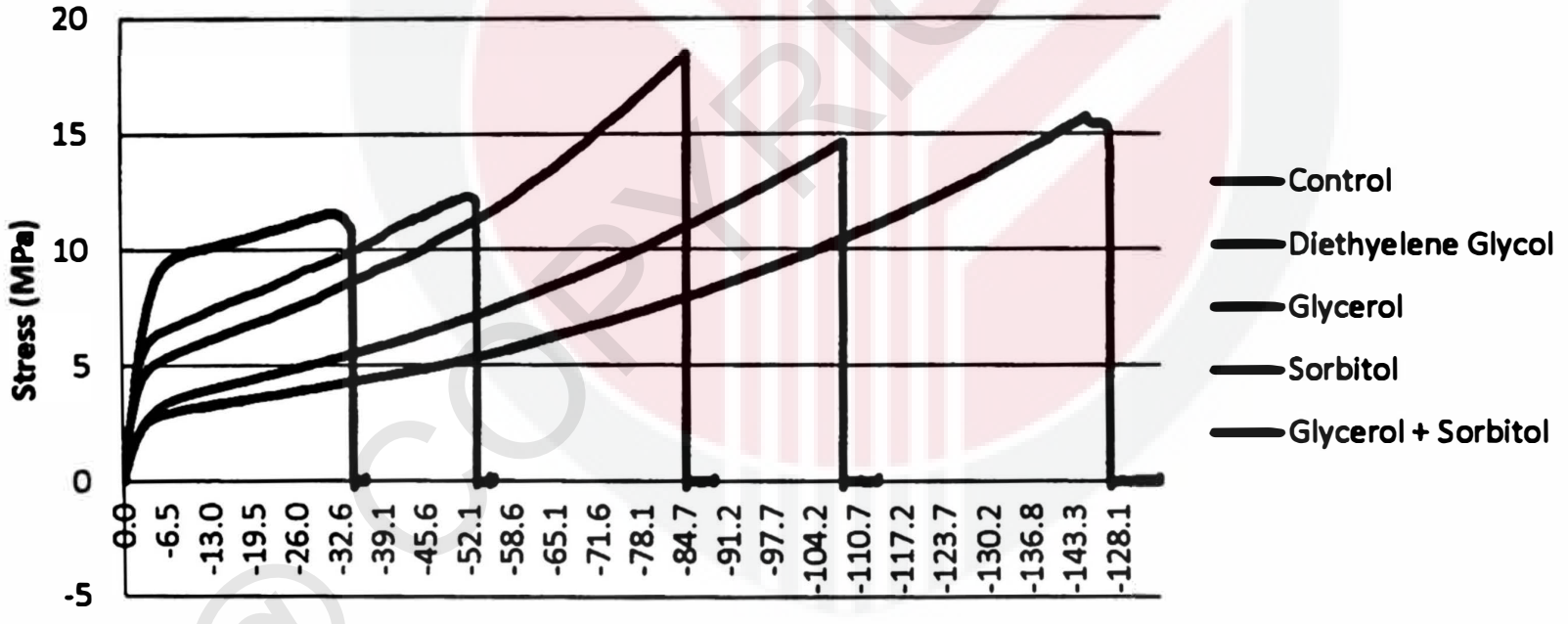


Figure 7: Stress vs. Strain of Different Plasticized Films

Stress vs. strain curves in Figure 7 above shown that the different types of plasticizer have brought different tensile and flexibility of the films. For control films which is the ones that is unplasticized or non any plasticizer were added in the films showing that the films behavior is so much brittle than any other, just like diethylene glycol. As for diethylene glycol, the

contribution of its brittleness is probably due to its little number in molecular weight structure which is only took about 106.12 g/mol (PubChem, 2016), this will lead to brittle state where the shorter number in molecular chain length have reduce its ability to entangled among each other, thus, breaking the molecular chain becoming easy.

The increasing over tensile strength and films flexibility can be observed as glycerol, sorbitol and combine glycerol and sorbitol were added to the films, due to the higher number of molecular weight of the plasticizer, the tendency of the plasticized films to deform have reduced. The higher number of molecular weight have contribute in higher number of molecular chain, thus, this will make the chains to form entanglement among each other and the bonds between the chain is hardly to break. As shown in Figure 7 above, the combination of plasticizer glycerol and sorbitol into the films have brought the longest deform moment which this meant by the elongation of the films is the highest one, this is due to the molecular number in the films structure had increase in the increase number of molecular weight of both plasticizer, thus, the flexibility of the films is also higher.

4.6.1 Tensile Strength of Fish Gelatin Plasticized Films

In order to ensure the quality and stability of the products, tensile test has been one of the test that are important to held which they are widely performed in almost every packaging industry. Tensile test had assist in determining the strength of material or in this case is film to resist the outward pull that they gain. A high tensile strength was the one which preferable for any packaging type especially when it comes to shipping a heavier products by pallet load, a stretch film with higher tensile strength is important as it will minimize the shipping purposes damage.

The effects of different types of plasticizer that added into fish gelatin based film on tensile strength is shown in Figure 3 below. Presence of glycerol (G) as plasticizer in fish gelatin based film have demonstrated high tensile strength with value of 18.309 MPa, second high amount of tensile strength with 15.381 MPa have been collected by Glycerol-Sorbitol (GS) combined plasticizer. For sorbitol (S) plasticized fish gelatin based film, as much as 14.908 MPa of tensile strength have been recorded, followed by control (C) which is un-plasticized film and di-ethylene glycol plasticized film has shown tensile strength value of 13.619 MPa and 13.043 MPa respectively. The possible reasons for the high tensile strength obtained by G-plasticized film is that the addition of glycerol improves the film strength due to the strong enough interaction occurs between glycerol and molecules obtain in fish gelatin, this will results to reduce the free volume and the mobility of molecules of the polymer itself, therefore, plastic film that was plasticized by glycerol have higher tensile strength than the others (Azadeh et al., 2014).

The tendency of reducing free volume and mobility of molecules stated above can be relate to the molar mass of glycerol which meant to be smaller, they contribute to the fasten easy interaction between glycerol-gelatin molecular chain effectively due to its size (Muhammed et al., 2015).

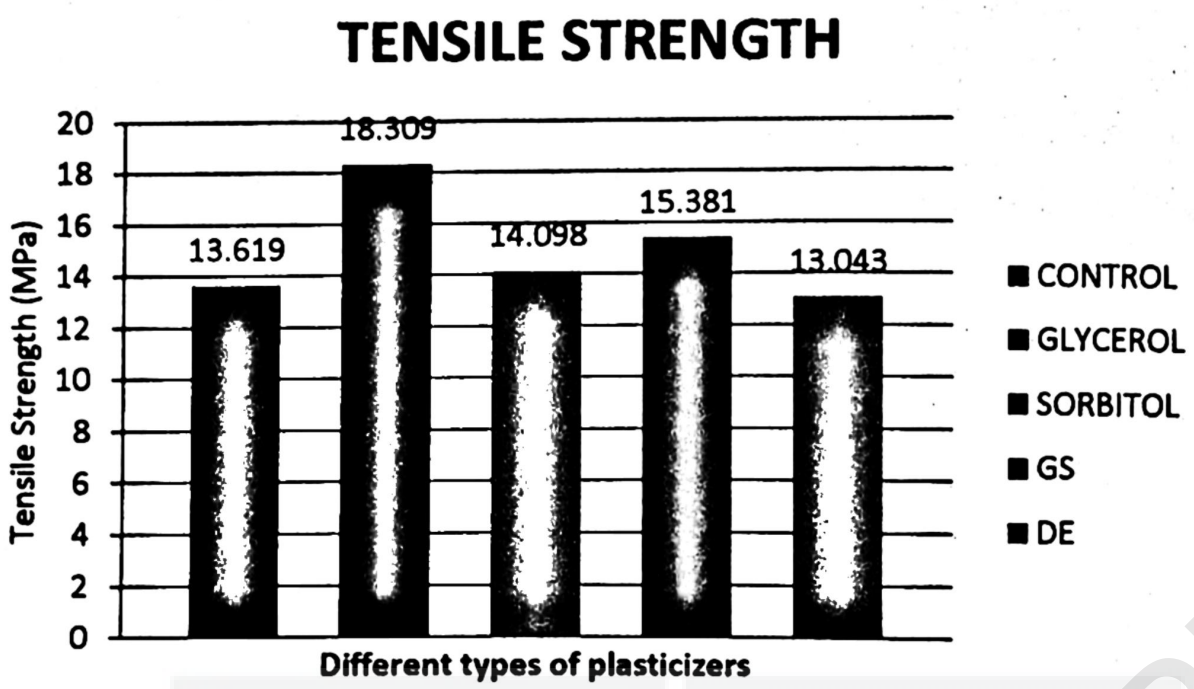


Figure 8: Tensile Strength on Plasticized Fish Gelatin based Films

4.6.2 Elongation at Break of Fish Gelatin Plasticized Films

Extendibility of film length from its initial to the point of break is what elongation at break meant. It is also known as fracture strain of ratio between increased length and its original length after breakage, it is the ability of films to deform before finally breaking or tearing (Muhammed et al., 2015). It is important the match flexibility of biopackaging film to its functional application and desired transportation include handling and storage of the package product to be specific, foods. To conclude, elongation at break shows the ductility of the films.

Graph of elongation at break with different type of plasticizer used on films has been attached as in Figure 4 below. As shown, films that were plasticized with combination of glycerol and sorbitol have the highest value of elongation before they deform which is at 140.276%. Regarding the tensile strength of GS-plasticized films, it shows less number than the G-plasticized films alone, this is due to the logic came where, tensile strength contribute to the strength of the films and elongation at break will contribute to the ductility of the films, while

GS-plasticized films is having less number of tensile strength this is because it is having higher ductility thus making it harder to deform.

The main reason of combined plasticized based film was having higher number of elongation at break can be explained in terms of their molecular weight and clearly shown in figure 7, low molecular weight of hydrophilic molecules such as glycerol and sorbitol could easily fit into the proteomic network and initiate the hydrogen bonding with active groups of amino acid residues which practically will reduce the protein-protein interactions. This condition of disruption and reconstruction of gelatin molecular chain will reduce the rigidity and promotes flexibility of films by allowing more chain mobility, thus, elongation at break is higher. The plasticizing effect of the components could be observed that the increasing of this properties can occurred as functions of the increase in number of plasticizers molecules in the films (Maira et al., 2006).

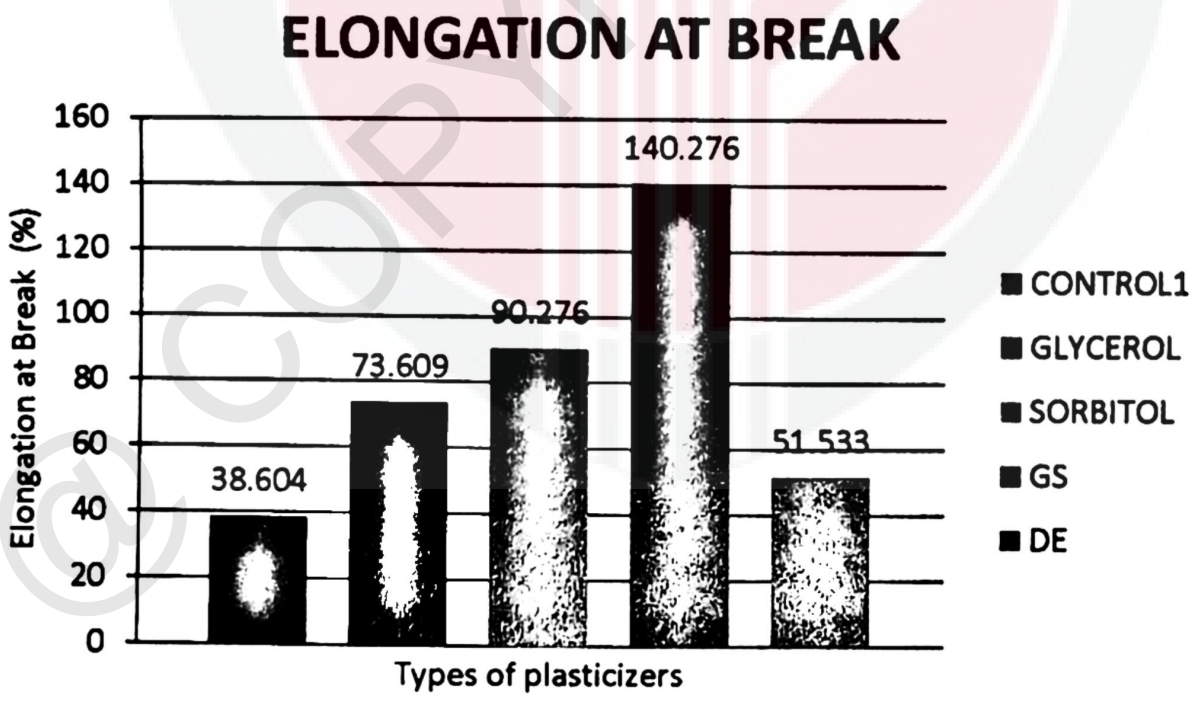


Figure 9: Elongation at Break on different plasticizer on fish gelatin based film

5.0 Conclusion

The characterization of fish gelatin plasticized based films have brought an effective effect towards the mechanical properties of the films, as the stronger the plasticizer bonds with gelatin molecules, the lower the glass transition temperature, plus the higher the tensile strength, also, the amount of weight loss would decrease as the stronger attraction makes the bonds even harder to break. The blends of plasticizers could be used in the production of edible films avoiding some problems with the use of a unique plasticizer. These research results were related to the molecular weight of the plasticizers, that is, the properties of the films were functions of the total number of plasticizer molecules present in the fish gelatin based films. Nowadays, starch based films have grown in name just like Polylactic Acid (PLA) usage as constituent to conventional plastics, this can be comparable as gelatin based film that is plasticized with combination of glycerol and sorbitol have brought even higher number in mechanical properties such as tensile and its elongation just as good as starch based films. According to (Muhammed et al., 2015) research, the tensile strength value of starch based films that is plasticized with combination of glycerol and sorbitol is 7.79 MPa, while for gelatin based films that is plasticized combination of glycerol and sorbitol have contribute to as much as 15.38 MPa with the same concentration of 30%. Moreover, starch based films gives 45% of elongation at break while gelatin based films make up till 140% of elongation at break in the same plasticizer combination used with the same concentration too. Thus, gelatin based films that are plasticized with combination of Glycerol and Sorbitol can be an alternative way in order to replace conventional plastics based on its suitable application.

6.0 Recommendation

There are several suggestions to be continued in further study for this research, it is recommended that the viability of bones from the same fish (tilapia), for gelatin extraction should also be investigated, as the bone that are produced by fish industry as wastes also have contributed to large amount of abundant waste and the bones of tilapia fish also are the sources of gelatin. Secondly, applied fish-gelatin based film to food packaging application such as laminate or wrapping the food with this type of film in order to investigate the behavior of foods and its endurance to protect the foods from bacterial or shortage of lifetime of the foods. As towards my view, it is easier to do application because it will expose us to the capability of the films to be marketable. Next suggestion is, casting by using maybe a uniform or bigger casting plate should be used to make the film more uniform in thickness and can be applied to application easily, also by having a larger size of film casting, defects part that contain bubble or insect can be discarded clearly to obtain an accurate results such as tensile and its elongation at break. Furthermore, as for the sticky properties of the fish gelatin, by using additive like oil and bee waxes can improve the properties. Finally, maybe try to do the compostable application on the fish-gelatin based film to prove the bio-degradability and its properties to degrade, this study will strengthen the view that fish-gelatin based film is really concerning on the earth saving things, due to its production is quite tough to be done, so by proving the bio-degradability may be worth to its production.

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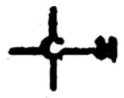
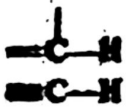
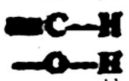
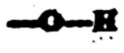
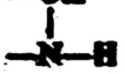
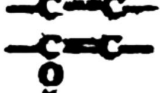
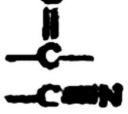
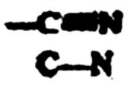
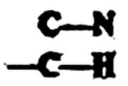
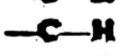
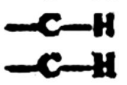
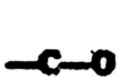
7.0 Appendices

Table 5: Structural Units and Absorption Frequencies. Retreived from:

http://www1.udel.edu/chem/fox/IR_lectureNotes.pdf

BOND		TYPE OF COMPOUND	FREQUENCY (CM^{-1})
	(stretch)	Alkanes	2800–3000
	(stretch)	Alkenes, aromatics	3000–3100
	(stretch)	Alkynes	3300
	(stretch)	Alcohols, phenols	3600–3650 (free) 3200–3500 (H-bonded) (broad)
	(stretch)	Carboxylic acids	2500–3300
	(stretch)	Amines	3300–3500 (doublet for NH_2)
	(stretch)	Aldehyde	2720 and 2820
	(stretch)	Alkenes	1600–1680
	(stretch)	Aromatic	1500 and 1600
	(stretch)	Alkynes	2100–2270
	(stretch)	Aldehydes, ketones, esters	1680–1740
	(stretch)	Nitriles	2220–2260
	(stretch)	Amines	1180–1360
	(bending)	Alkane	1375 (methyl)
	(bending)	Alkane	1460 (methyl and methylene)
	(bending)	Alkane	1370 and 1385 (isopropyl split)
	(bending)	$R-CH=CH_2$	1000–960 and 940–900
	(bending)	$R_2C=CH_2$	915–870
	(bending)	*cis* RCH=CHR	790–650
	(bending)	*trans* RCH=CHR	990–940
	(out-of-plane bending)	*mono* subst. benzene	770–730 and 710–690
	(out-of-plane bending)	*o*-subst. benzene	770–735
	(out-of-plane bending)	*m*-subst. benzene	810–750 and 710–690
	(out-of-plane bending)	*p*-subst. benzene	860–800
	(stretch)	Primary alcohol	1050–1085
	(stretch)	Secondary alcohol	1085–1125
	(stretch)	Tertiary alcohol	1125–1200
	(stretch)	Phenol	1180–1260

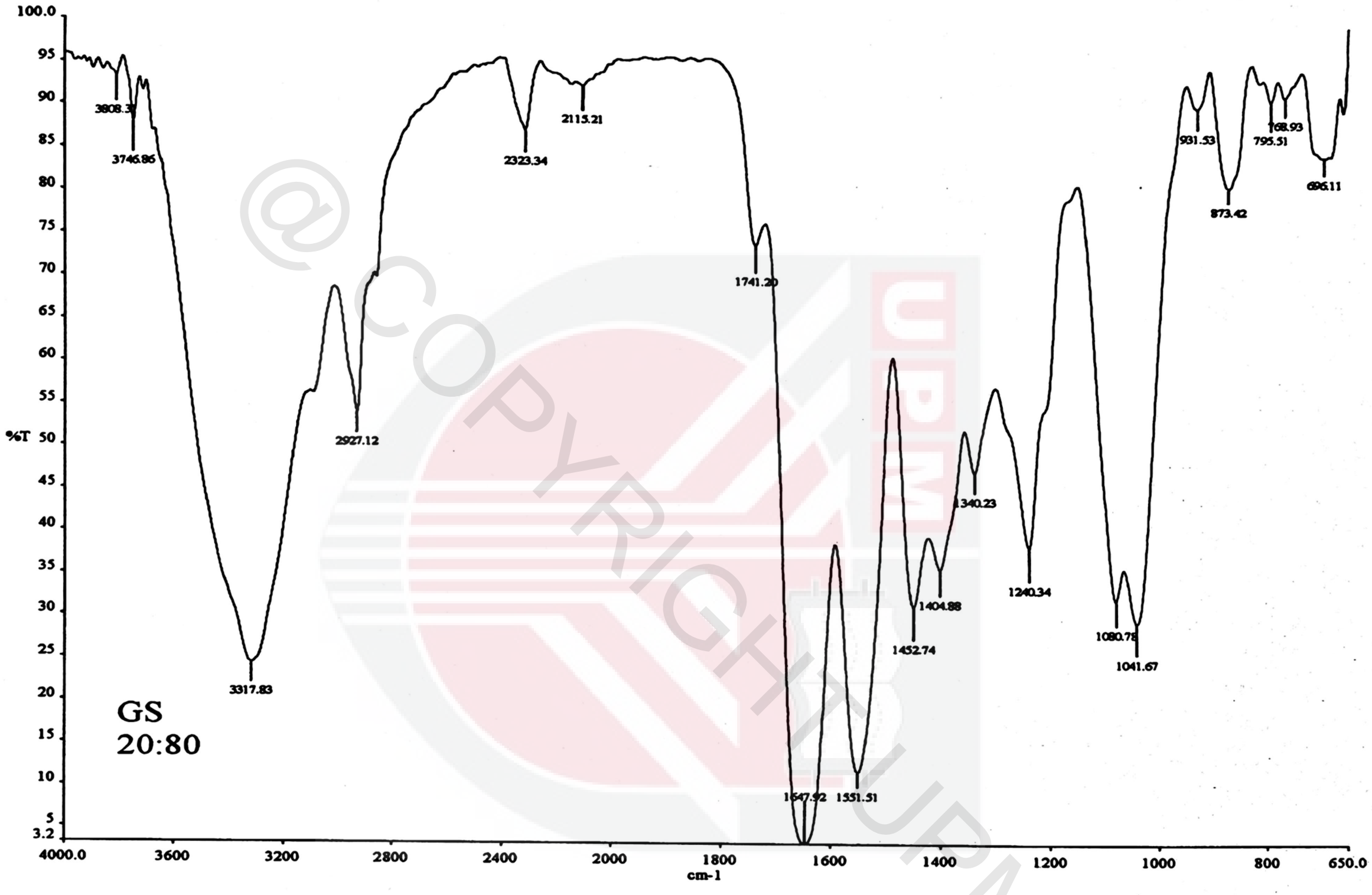


Figure 10: FTIR results on (GS)-plasticized films

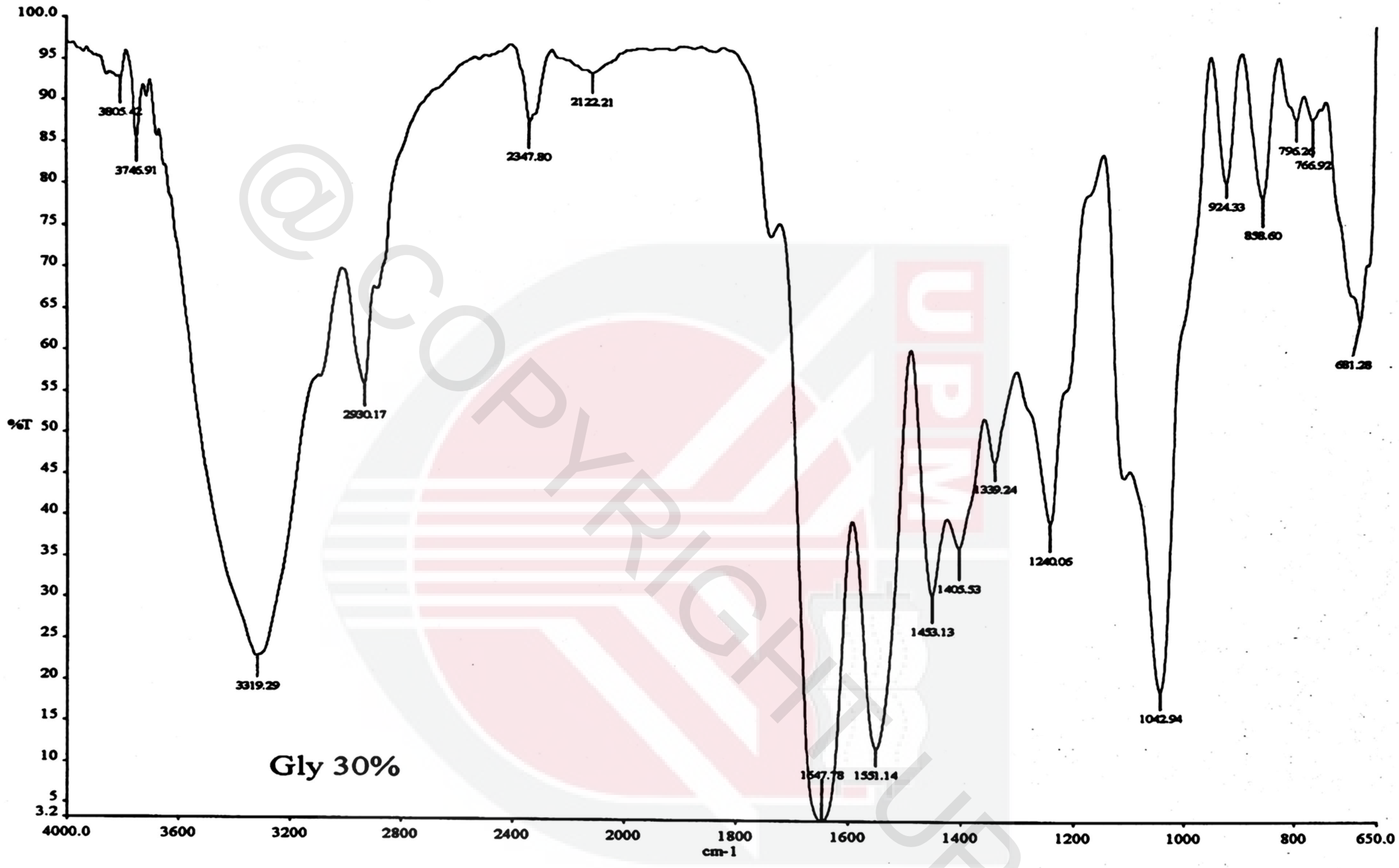


Figure 11: FTIR results based on G-plasticized films

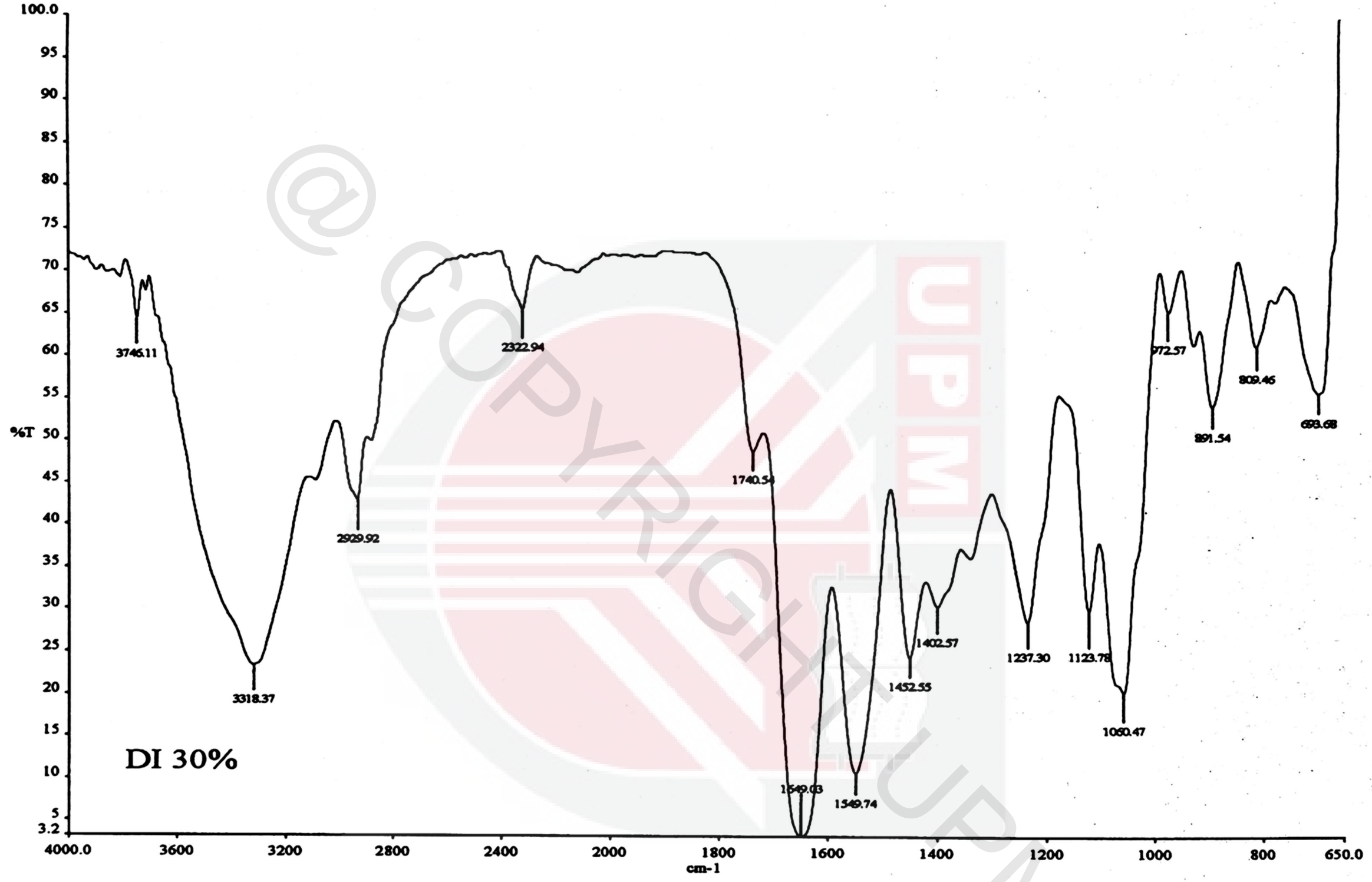


Figure 12: FTIR results on DI-plasticized films

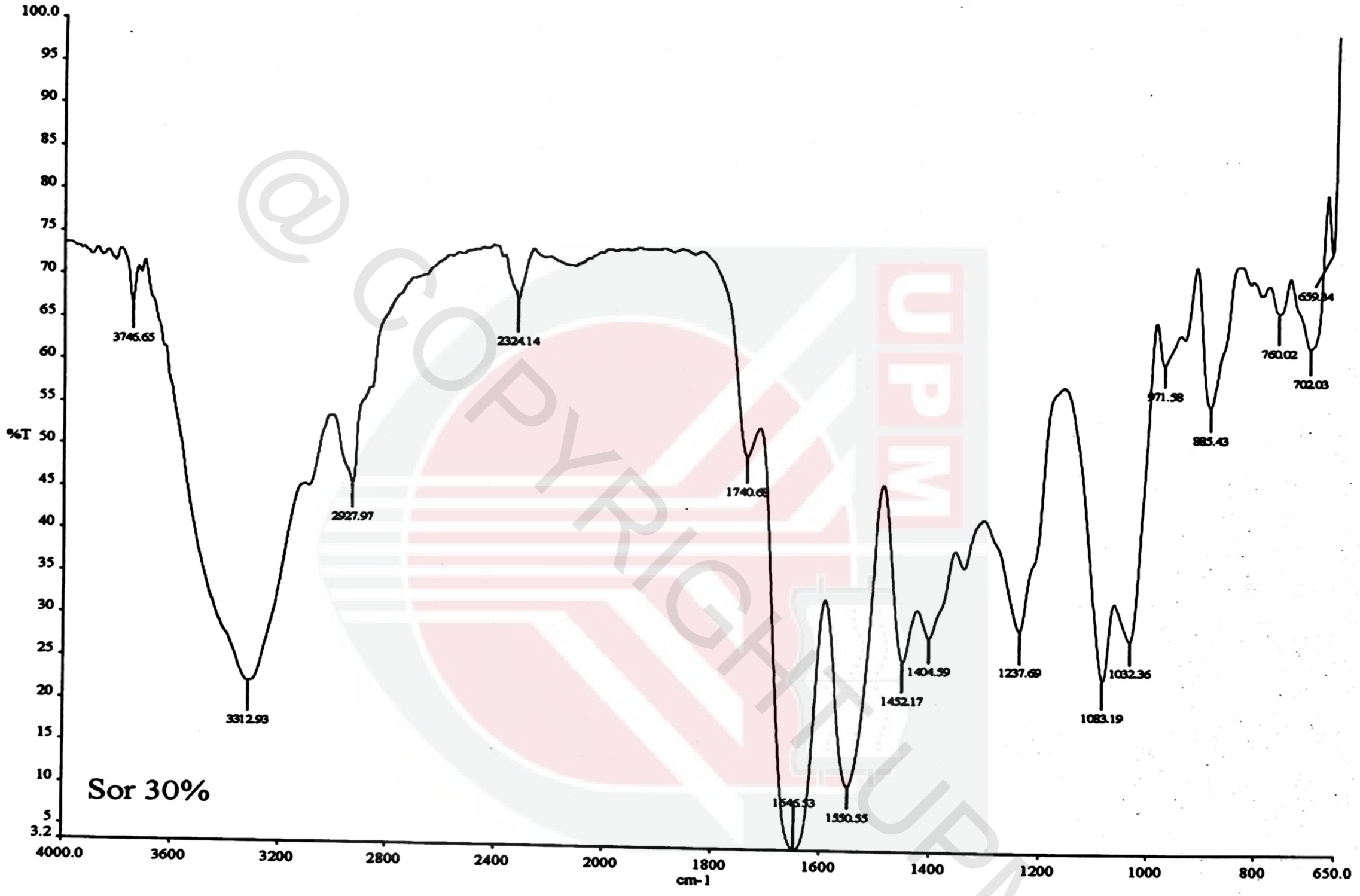
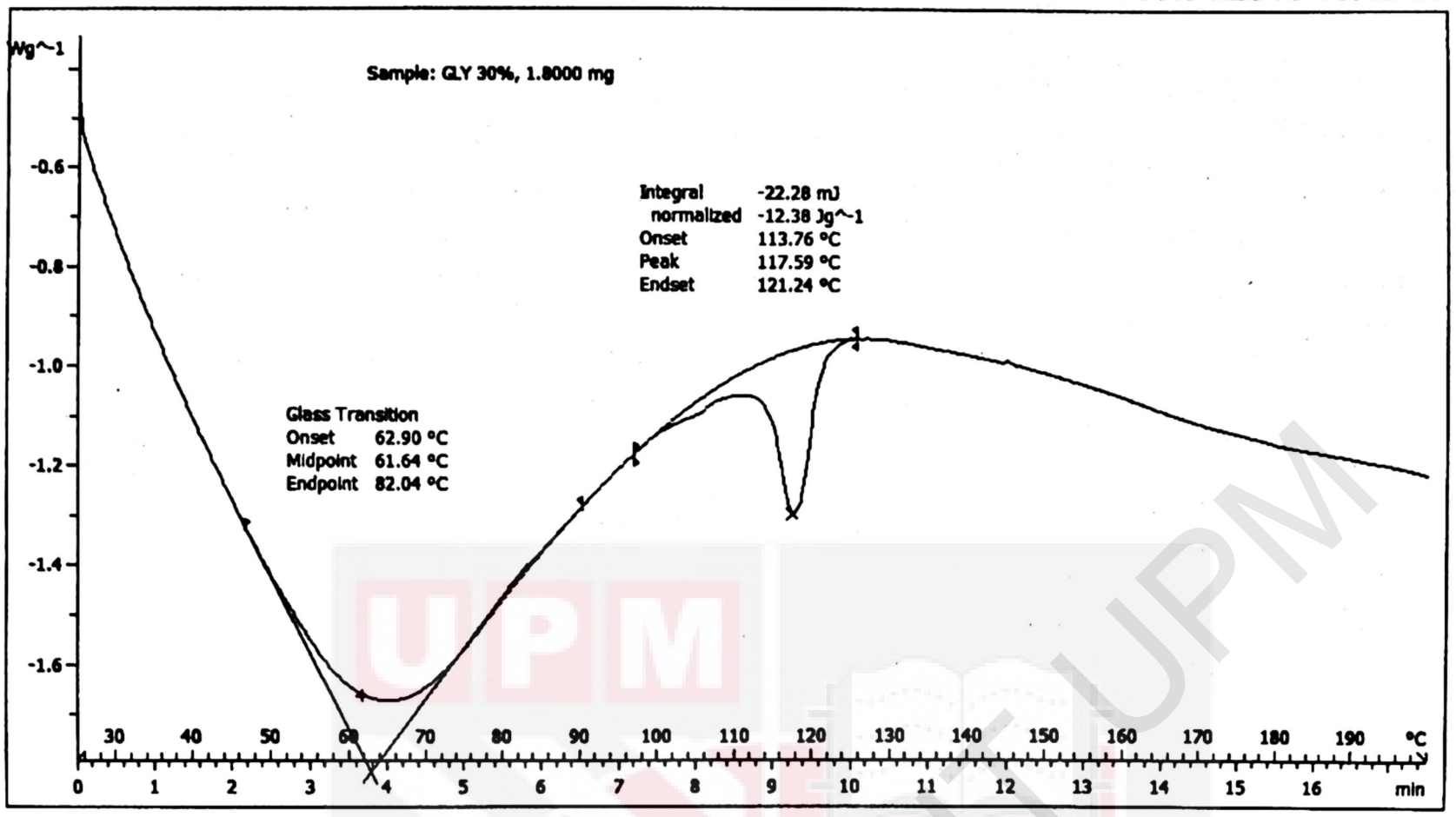


Figure 13: FTIR results on S-plasticized films

^exo

GLY 30%

30.04.2019 15:12:30



DEMO Version

STAR° SW 12.10

^exo

SOR 30%

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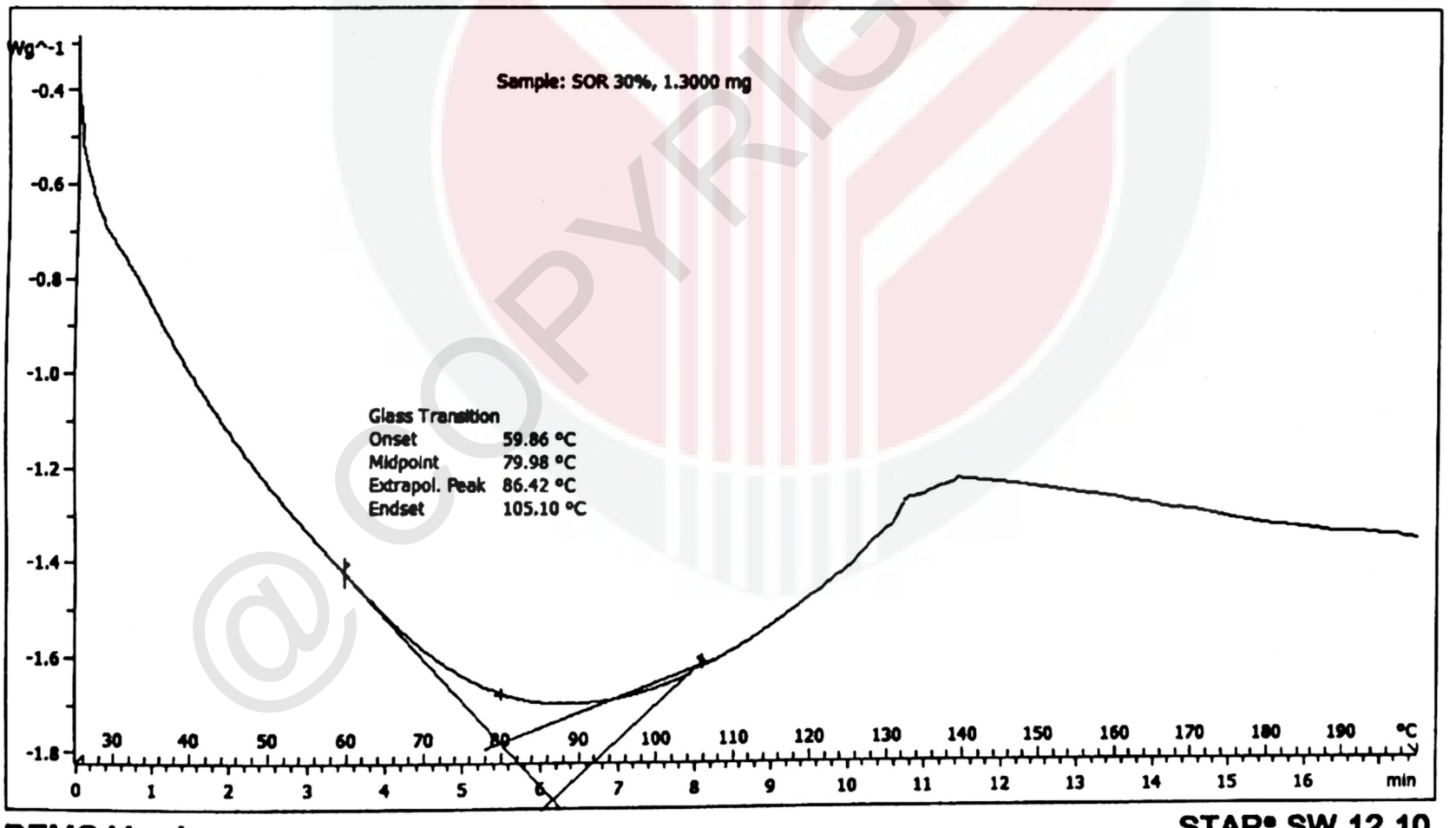
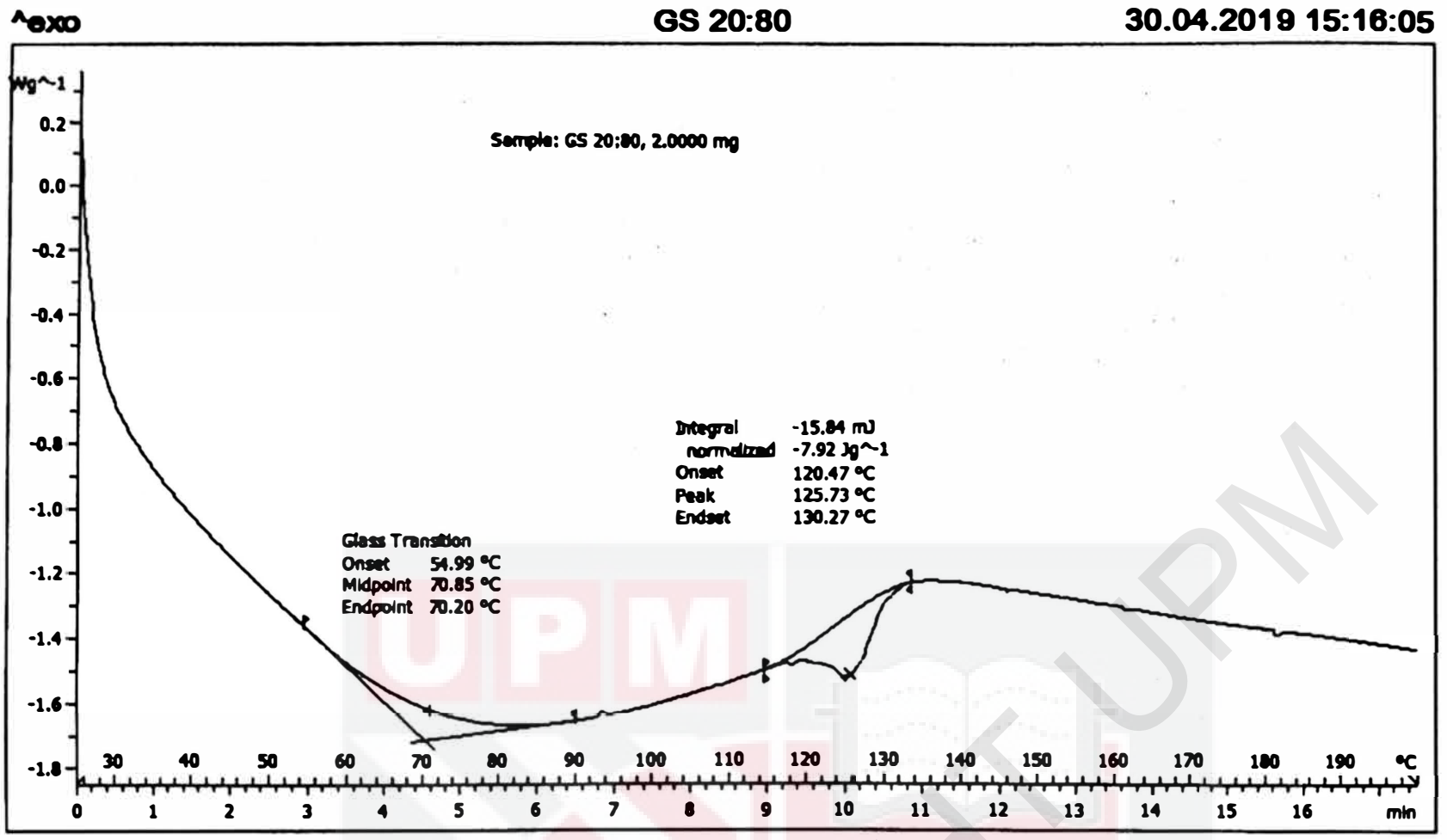


DEMO Version

STAR° SW 12.10



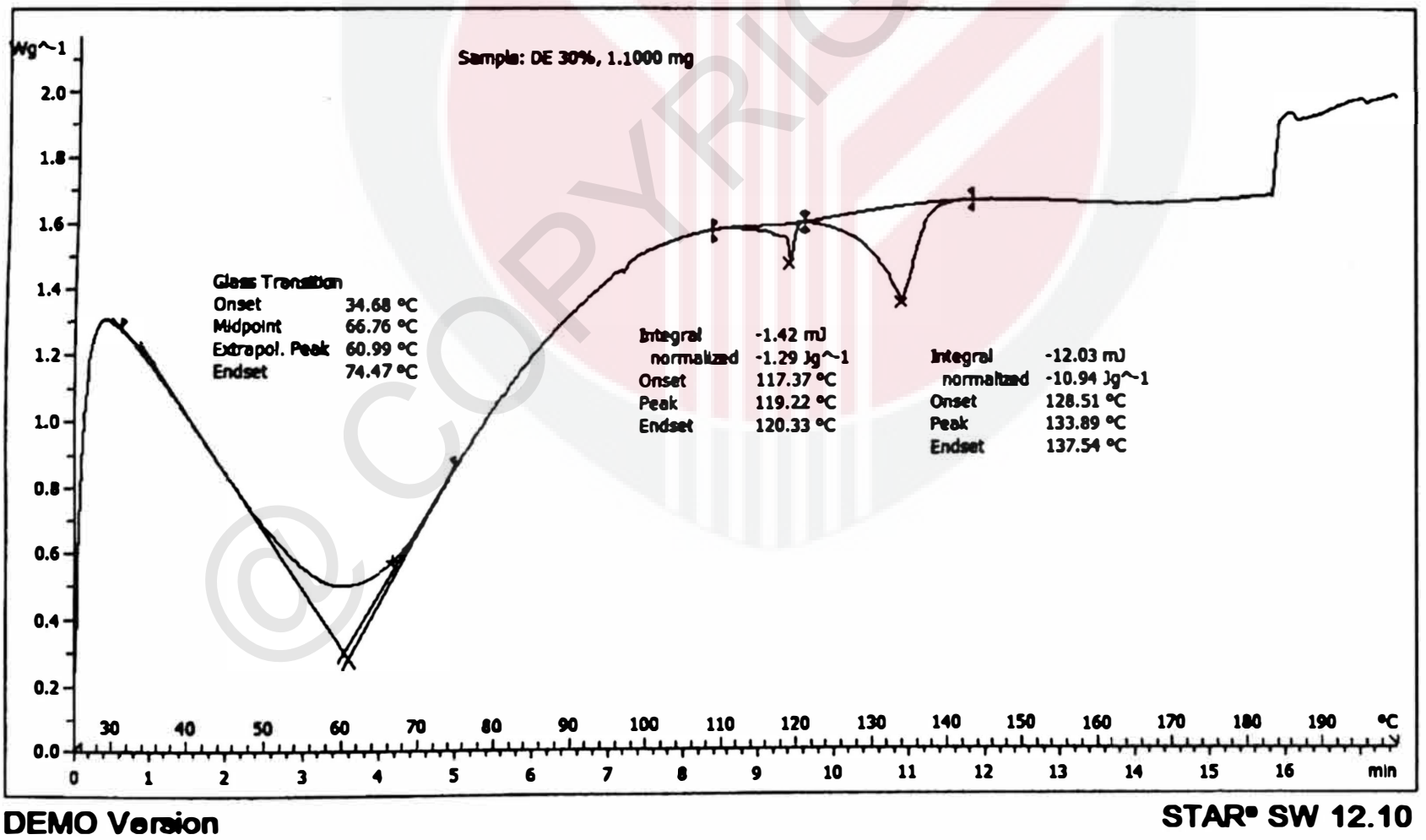


Figure 14: DSC results on different type of plasticized films