Università degli Studi di Modena e Reggio Emilia
Facoltà di Ingegneria "Enzo Ferrari"

PhD SCHOOL in
Industrial and Environmental Engineering

Characterization and applications of PLA/Date polyphenol blends

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ABSTRACT

Date palm is one of the oldest trees in the gulf region. The scientific name is *Pheoenix dactylifera L*. Date fruit are highly popular in the Middle East and North Africa. It plays an important part in the social life of the people. Nowadays Qatar’s production of date fruit is around 71% of its total agricultural crop. This is due to suitable weather for this type of tree and due to the high consumption per capita. It is consumed as fresh dates or as sundried dates during one year. The expired date fruit or the wrong way of preservation lead to discarding the dates that be used as organic fertilizer. The valorization of discarded date palm fruit is also important for the economy of Qatar. Since the dates are a rich source of the polyphenols, many studies discussed the uses of polyphenols as antioxidant, antibacterial or as natural stabilizer in industrial applications. The structure of polyphenol composed of a single is ring in a simple form as phenolic acid or more than one aromatic ring attached with hydroxyl groups, such as flavonoids. Polyphenols can interact via electron donation and resonance stabilization. Because of these advantages of polyphenols, many studies discussed the effect of the polyphenol blends with polymers, but no studies reported on the effect of polyphenols extracted from date palm fruit blended with poly(lactic acid).

Poly(lactic acid) (PLA) is a thermoplastic polymer which is derived from renewable resources. It is one of the highest consumption bioplastics in the world compared to petro-chemical based polymers due to a wide range of commodity applications. PLA has some weaknesses such as weak thermal stability, impact and low ductility; therefore many research studies are focusing on PLA modification for extending PLA applications. Thus, adding modifiers is essential to enhance its properties.

In this research, a set of PLA/polyphenol extracted from date palm fruit (DPF) were prepared for medical and industrial applications. The results were compared with those on composites prepared with PLA mixed with commercial polyphenols extracted from green tea leaves. The first blends were prepared by electrospinning to be used as cell culture scaffolds for tissue engineering applications. The results revealed that scaffolds became more hydrophilic with addition of polyphenols, and it was found that both cell proliferation and cell viability were enhanced with increased polyphenol concentration within the scaffolds.

The second part of the research reported a comparative study between polyphenol extracted from date palm fruit and green tea leaves polyphenols. Different concentrations of both polyphenols were prepared through melt mixing followed by compression molding, and the morphology as well as mechanical and thermal and properties of the blends/composites were investigated. It was found that the addition of polyphenols led to an improvement in the thermal stability, with the green tea polyphenols giving a better improvement in thermal stability due to the existence of tannic acid. The Young’s modulus and tensile strength were found to decrease, and the elongation at break were found to increase. The glass transition temperature were obtained from DMA and showed a broadening in the tan δ peak, and the glass transition temperature (T_g) slightly decreased after the addition of the polyphenols, which confirmed that the polyphenols increased the free volume of the PLA. The solid polyphenol particles, extracted from green tea leaves, also acted as a nucleating agent for PLA crystallization.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Polyphenols are a widespread group of secondary metabolites found in all plants, representing the most desirable phytochemicals due to their potential to be used as additives in medical field, food industry and others fields. The quality and quantity of phenolic compounds in plants are influenced by the parts of the plant, the stage of growth and the environmental growing conditions. The interest in phenolic compounds has been increased over recent years, particularly because they are excellent antioxidants ,stabilizer and compatibilizer. Consumption of antioxidants has shown its efficiency in the prevention of cardiovascular diseases, cancer and against skin aging [1,2].

Date palm tree are important crops in the Middle East generally and in the Gulf region specifically. It plays an important role in the economic and social life of the people, and their products are extensively used in daily life [3,4]. The date fruit contains fat (0.20–0.50%), proteins (2.30– 5.60%), dietary fiber (6.40–11.50%), minerals (0.10–916 mg/100 g dry weight), vitamins (C, B1, B2, B3, and A) and very little or no starch. Date fruit is an rich source of polyphenols [4]. It can be consumed as fresh dates or as sundried dates.

Date fruits ripen through five different stages *Habauk*, *Kimri*, *Khalal* (*or Bisr*), *Rutab*, and *Tamr* as shown in fig. 1.1

Figure 1.1 Different stages of ripening of date fruit.

Different region give different cultivars of date palm fruit, which vary in shape, size, and weight. In addition, they can vary in total phenolic content. Lemine et al. [6] reported that the total flavonoid content decreases significantly from the Kimri stage, which is the second stage. Another study showed that the flavonoid content increases during the maturity stages from Khalal to Tamr [7]. This means that the mature stage of date fruit is the best stage for polyphenol extraction. In this research, we used the last stage of discarded date fruit Tamr stage. Phenolic compounds are a large class of plant secondary metabolites, showing a diversity of structures, from rather simple structures, e.g. phenolic acids, through polyphenols such as flavonoids that comprise several groups as shown in the classification Fig1.2

There is no universal procedures for extraction polyphenols from plants, but there are some factors that may affect the total phenolic content. Shadadi et al. [8] showed that that the drying process affect the total phenolic content (TPC), which varied with temperature and decreased by increase the drying temperature.

Many available methods of quantification of total phenolic content in biological samples are based on the reaction of the phenolic compounds with a colorimetric reagent, which allows measurement in the visible portion of the spectrum. The Folin–Ciocalteu (F–C) assay is such a method, which has been proposed as a standardized method to measure the total phenolic content. The antioxidant activity of polyphenols evaluated by the DPPH_ method and investigated by many researchers. the structures of these phenolic compounds allow for the donation of hydrogen atoms protons to free radicals, thus inhibiting the oxidative process. [8].

Polyphenols are natural compounds with aromatic structures containing one or more aromatic rings with or without the vicinity of a heterocycle and which are grafted with hydroxyl, carboxyl, methoxyl and carbonyl functional groups. According to the biological function, polyphenols can be classified into different classes; however, two main groups of polyphenols can be identified: the flavonoids and the non-flavonoids.

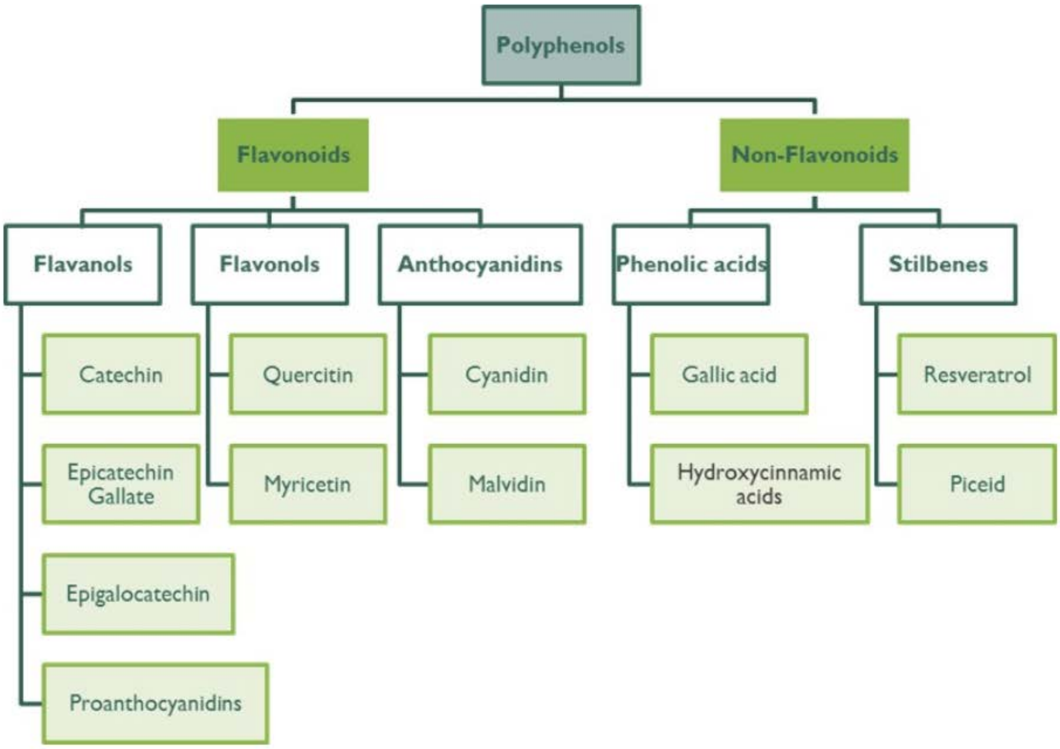
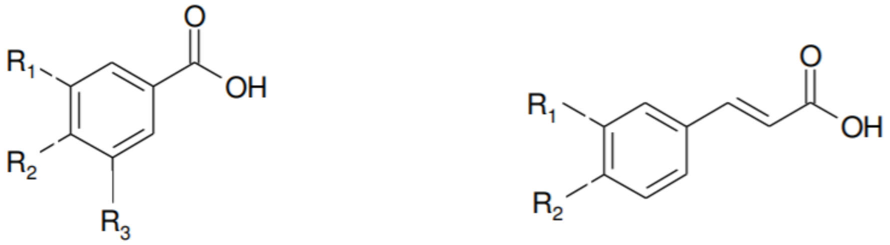


Figure 1.2 Classification of polyphenols [10]

Non-flavonoids can be classified according to their chemical structure into the following groups: phenolic acids with the subclasses derived from hydroxybenzoic acids and from hydroxycinnamic acid, stilbenes, lignans and the polymeric lignins.

Phenolic acids from a chemical point of view, phenolic acids containing carboxyl group with one or more hydroxyl groups grafted onto a benzene nucleus. Phenolic acids are the most abundant polyphenols in our diets (30%) and are found in different forms in plants, including aglycones (free phenolic acids), esters, glycosides, and/or bound complexes. Based on position of the hydroxyl group, phenolic acids can be divided into two main types, benzoic acid (C1– C6) and cinnamic acid derivatives (C3–C6) as shown in figure below.



p-hydroxybenzoic acid (R_2 - OH)
Gallic acid (R_1, R_2, R_3 - OH)
Vanillic acid (R_2 - OH, R_3 - OCH_3)
Syringic acid (R_1, R_3 - OCH_3, R_2 - OH)
Hydroxybenzoic acids

Ferulic acid (R_1 - OCH3, R_2 - OH)
Caffeic acid (R_1, R_2 - OH)
Hydroxycinnamic acids

Figure 1.3 Basic structure of non-flavonoids

Flavonoids are the most abundant polyphenols in our diets. One or more hydroxyl groups of phenols are combined with reducing sugars. Flavonoids are also associated with a wide range of biological effects on health, including antibacterial, anti-inflammatory, anti-allergic and antithrombotic. The term flavonoid is assigned to the polyphenolic compounds of the general structure C6- C3-C6 in which the two phenolic benzene rings A and C are linked by a pyran ring B

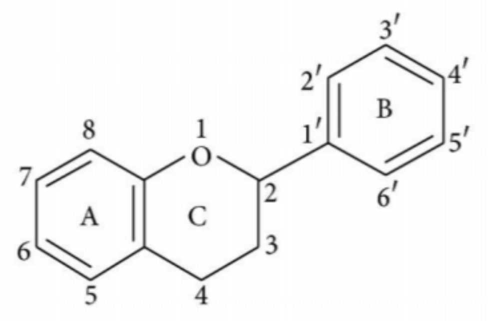


Figure 1.4 Basic structure of flavonoids

Flavonoids are divided into subgroups: flavonols, flavanols, and anthocyanins. Flavonol is abundant in date palm fruit, onions, broccoli, and in green tea, grapes and several fruits. Many researchers reported the incorporation of polyphenols with polymer matrix and reported the effect of polyphenols as good stabilizer with antioxidant and UV protection with PP, The chemical modifications of polyphenols have been investigated to improve its compatibility with the hydrophobic polymer matrix such as PLA .

Several researchers reported the blend of polyphenols with different polymer matrix; Ambrogi et al [2] studied the effect of polyphenol extracted from wine waste on polypropylene stability, the results indicated enhancement in thermal stabilization. Another study done by Misso et al. investigated the potential impact of the tannin as compatibilzer in polypropylene in wood plastic composite industry WPC, While condensed tannin extracts are constituted by oligo-/polyphenols, which maintain similar composition, and therefore is possible to have a more reliable resource for compatibilizing the WPCs [3]

In the last decades, many research studies focused on biodegradable polymers due to their ability to biodegrade when disposed in the environment [4,5]. PLA is a bio-based material and is used extensively in medical, packaging and industrial applications [6-5] because of its good strength and moderate barrier properties. It is derived from renewable plant resources such as corn starch and sugar. Although it has good properties, it has a weak thermal stability and a weak resistance to the environment, because its relatively low glass transition temperature of 60 °C can cause it to rapidly lose its structural integrity and deformation under load is a problem at high temperatures [7], which makes it unsuitable for many applications. A number of research efforts focus on the modification of the polymer matrix by the addition of modifiers to enhance the thermal stability, impact strength and durability. PLA is one of the most promising biopolymers. It is a biodegradable thermoplastic polyester that is manufactured by biotechnological processes from renewable resources such as corn, starch sugars, cassava or sugarcane [11-15]. PLA can look and behave like polyethylene (used in plastic films, packaging and bottles), polystyrene (Styrofoam and plastic cutlery) or polypropylene (packaging, auto parts, textiles). PLA is a high-strength, high-modulus polymer, and it ranges from amorphous polymers to semi-crystalline polymers with melting points of 160-180 °C. PLA has a glass transition temperature around 60–65 °C, a melting temperature 160-180 °C, and a tensile modulus of 2.7–16 GPa [16]. PLA is one of the most exploited scaffold material for tissue engineering applications [7, 8].

Tissue engineering (TE) combines cells, materials and appropriate biochemical factors to produce live tissues that would replace degenerative tissues. Despite the significant potential applications, developments in this field are far beyond the expectations and there are only few tissue-engineered products in the market [1]. One important component in TE is the selection of appropriate biomaterial for scaffold production that would not be rejected by the immune system once implanted to the host. Thus, many researchers work on techniques to promote tissue regeneration in biological environments [2]. The process requires overcoming inhibitory factors and promoting cell adhesion and cell proliferation to make the engineered tissue functional. Usually, biodegradable scaffold materials are selected to enhance tissue regeneration for replacing the synthetic biomaterial with the extracellular matrix (ECM) produced by the cells [3]. Another major challenge is the formation of reactive oxygen species (ROS) on the surfaces of transplanted biomaterial scaffolds [4]. In the body, ROS forms as a by-product of normal oxygen metabolism and is associated with important roles in cell signalling, wound healing and inflammatory responses. ROS oxidizes and releases molecules such as hydrogen peroxide (H_2O_2), superoxide anions (O^{-2}) and free radicals including hydroxyl (OH•) and peroxy (R-COO•), which are usually short-lived and highly reactive inside the body. During diseased states such as inflammation, diabetes and cancer, ROS levels can increase dramatically which can damage the structure of the cells [5]. Thus, the use of antioxidant materials blended with biomaterials is an essential strategy in TE to prevent excessive ROS production, which might trigger inflammation and eventual rejection of the implant. Along with other biodegradable polymers such as polycaprolactone (PCL) [6] and polyvinyl alcohol (PVA), polylactic acid.

Recent investigations on PLA scaffolds have focused on the optimization of their cell adhesion properties [9]. For instance, Llorens et al. [10] studied the incorporation of different types of antioxidants on PLA electrospun fibre scaffolds and found that the addition of antioxidants enhanced the cell adhesion characteristics. Moreover, it has been reported within in vitro and in vivo settings that when antioxidants are provided, free radicals responsible for cell damage are attenuated which results in rapid wound healing due to enhanced proliferation [11]. Therefore, incorporation of polyphenol antioxidant to PLA scaffolds, as reported herein, is expected to improve biocompatibility of these scaffolds in TE applications. Polyphenols extracted from plants have been used as dietary antioxidants and recent investigations showed that these compounds provide protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [12]. Several studies discussed the methods of polyphenol extraction from different plants such as green tea [13] and grapes [14]. Yanna et al. [15] investigated the antibacterial effect of different loadings of tea polyphenol on PLA electro spun nanofibers and showed enhanced antibacterial activity with addition of polyphenol. However, antibacterial activity gradually gets impaired when tea polyphenol content surpasses a certain level. Date palm is another plant containing polyphenols [16] and it is widely abundant in the Middle East [17]. Although there are several studies for the application of PLA scaffolds in TE [10], to best of our knowledge, there is no study that discussed the effect of the derived polyphenols from date palm fruit in PLA scaffolds.

In chapter 2, we introduce new electro spun nanofiber scaffolds made from PLA that was blended with polyphenol extracted from date palm fruits. The electro spun nanofibers were characterized for their physical, mechanical and morphological properties. In vitro biocompatibility tests were performed using NIH/3T3 fibroblast cells, since fibroblasts are the most abundant cells in skin tissue. MTT, live/dead and cell migration assays were performed for biological characterization. The aim of the present work is to demonstrate the potential benefits of incorporating polyphenol extracted from date palm fruit in PLA scaffolds for TE applications.

Chapter 3 compares the efficiency of date palm polyphenol extract with a commercial green tea polyphenols on the stability of the polylactic acid, however, the tea is one of the most popular beverages consumed in the worldwide. It is from the plant *Camellia sinensis* and consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea. Green tea contains polyphenols, which include flavonoids and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols (GTPs) are flavonols, commonly known as catechins.

Byun et al. [57] studied the effect of different additives, including polyphenol, on the physical and mechanical properties of PLA and observed a decrease in the T_g and an increase in the elongation at break, indicating the plasticizing effect of the polyphenol. Other studies [9,10] showed that polyphenols in polymers acted as antioxidants anticancer agents, thermal stabilizers and plasticizers.

In this study polyphenols were used from agro waste of date palm fruit industry which as a rich source of polyphenols . Date plant (*Phoenix dactylifera L. Arecaceae*) fruits are important crops for most of the population in Middle Eastern countries, and most of these fruits are produced in the Arab World (> 80%). This fruit is important from nutritional and economic viewpoints. the total phenolic content depends on different factors, e.g., maturity date. Studies done by Hong et al. [59] showed that date palm fruits at their different stages of maturity contain thirteen classes of polyphenol such as flavonoids, flavones, etc. The polyphenols also have a high antioxidant capacity [60]. In this work, a comparative study done to investigate the stabilization effect of polyphenols derived from two source of polyphenols such as date palm fruit and green tea leaves.

Green Tea (Camellia sinensis L.) is the second most consumed beverage in the world and characterized by its high polyphenol content, In this part, we compared the influence of different concentrations of polyphenols, extracted from respectively green tea leaves and date palm fruit, on the thermal stability and mechanical properties of PLA.

1.2 Problem statement

Today worldwide production of dates are continuously increasing since date fruits have gained great importance in human nutrition due to their rich content of essential nutrients. Daily tons of date palm fruit wastes are discarded by the date processing industries leading to cost and effort consuming. Wastes such as date pit/wrong preserved fruit represent an average of 10% of the date palm industry. Thus, there is an essential need to find suitable applications for this waste. Valorization and using agro waste as a source of polyphenols is valuable point in date palm industry. Since the date fruit is a rich source of polyphenols with great properties such as good stabilizer, high antioxidant properties and bio- compatibilizer.

Incorporation of polyphenol extracted from date palm with biodegradable polymer matrix such as PLA is a good choice that face the pollution expanding due to the increase in the production of synthetic plastics from crude oil. It is important to replace the conventional petro-chemical products with eco-friendly and sustainable plastic such as PLA. Thus, there are many efforts to introduce agro-industrial wastes into bioplastic for different applications. In this research PLA is being used as matrix for polyphenols extracted from date palm fruit , for biomedical application and the second part of the thesis discussed the efficiency of polyphenols on PLA stability compared with green tea polyphenols. To the best of our knowledge, no similar study was reported in the open literature.

1.3 Objective of the study

The overall objective is to develop a green sustainable biopolymer based on polyphenols extracted from date palm fruit and its blend with a biodegradable polymer such a PLA, and to study its effect on different PLA applications. Commercial polyphenols, extracted from green tea leaves, was also studied in a similar way in order to compare our ‘new’ date palm fruit polyphenols with commercially available polyphenols.

1) In Chapter 2 we investigated the effect of polyphenol on scaffold for tissue engineering applications.
2) In Chapter 3 we investigated the effect of different concentrations of polyphenols from two sources such as date palm fruit and green tea on the thermal and mechanical properties of PLA.

CHAPTER 2

ELECTROSPUN POLYLACTIC ACID/DATE PALM POLYPHENOL EXTRACT NANOFIBRES FOR TISSUE ENGINEERING APPLICATIONS

This chapter has been published in Emergent Materials 2019; 2:141-151 (https://doi.org/10.1007/s42247-019-00042-8)

In this part of study, a set of polylactic acid (PLA)/polyphenol extracted from date palm fruit (DP) blends were prepared by electrospinning process to be used as cell culture scaffolds for tissue engineering applications. for this purpose, PLA/DP blends with variable composition were dissolved in dichloromethane/dimethylformamide (70:30, v/v) mixture and then electrospun to obtain the fibres. contact angle measurements, dynamic mechanical analysis, mechanical tensile and scanning electron microscopy (SEM) tools were used to study the physico-mechanical properties of the electrospun scaffolds. The results revealed that scaffolds became more hydrophilic with addition of dp. increasing the polyphenol concentration caused the tensile strength and young’s modulus to decrease. the SEM graphs indicated a decrease in fibre diameter with increasing dp content. in addition, it was found that both cell proliferation and cell viability were enhanced with increased dp concentration within the scaffolds. the scratch test shows that there is an enhancement in cell migration through the scratch for PLA/DP scaffolds; again, higher dp content resulted better migration. our results suggest that improved mechanical properties, decreased fibre diameter and enhanced hydrophilicity with addition of dp improved cell migration and cell adhesion for the scaffolds. overall, these results demonstrate that dp is a potential natural cell-friendly product for tissue engineering applications such as tissue regeneration or wound healing assays.

2.2 Materials and methods

2.2.1 Materials

The polylactic acid (PLA) used in this study is a high molar mass biopolymer (Ingeo™ Biopolymer 2003D) obtained from NatureWorks, LLC (USA). It is transparent with a density of 1.24 g cm−3 and melt flow index of 6.0 g/10 min at 2.16 kg/210 °C. Dimethyl formamide (DMF) (ACS, 99.8%), gallic acid and sodium carbonate were purchased from Sigma Aldrich. Dichloromethane (DCM) with a density 133 kg/L and Folin-Ciocalteu reagent were obtained from BDH Middle East LLC. All reagents were used as received without further purification.

2.2.2 Polyphenol extraction from the data palm fruits

Extraction of polyphenol from date palm fruits was conducted according to the steps shown in Figure 2.1.

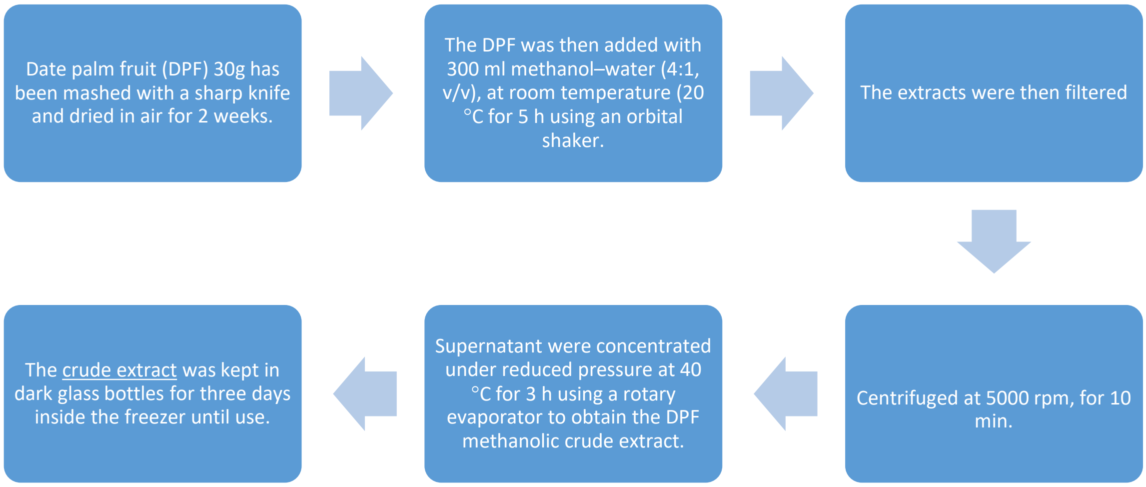


Fig. 2.1 Schematic diagram of polyphenol extraction methods from date palm fruit.

2.2.3 Production of PLA/polyphenol blend by electrospinning

7 wt% PLA was dissolved in DCM:DMF mixture (70:30) for at least 3 h at room temperature. Polyphenol was then added to the PLA solution and the mixture was ultrasonicated for 20 min. Three different sets were prepared with different polyphenol concentrations as shown in Table 2.1. The electrical conductivity of the solution was measured using a conductivity metre (Mettler Toledo S470). Viscosity and conductivity characteristics of the blends are summarized in Table 2.2. Electrospun nanofibres were fabricated using NaBond electrospinning machine (Shenzhen, China). PLA suspensions were loaded into a 10-mL syringe, with a bluntend, stainless steel needle 0.7. An aluminium foil covered rotating drum collector was used as the collection screen. The collector is connected to the ground electrode of the power supply and the distance between the screen and the needle tip was 15 cm. The electrospinning process was conducted at room temperature at a voltage of 13 kV, flow rate of 0.8–1.5 mL/h and drum speed of 500 rpm. The thickness of produced mats was around 50 µm.

2.2.4 Characterization methods

2.2.4.1 Polyphenol characterization

The total phenolic content was determined using Folin-Ciocalteu reagents. Forty microliters of extract was mixed with 1.8 mL of Folin-Ciocalteu reagent (diluted with 10- fold with distilled water) and the solution was kept at room temperature for 5 min. Then, 1.2 mL of sodium carbonate solution 7.5 wt% was added to the mixture and the solution was kept at room temperature for 60 min. The absorbance was then measured at 650 nm using a UV/VIS Biochrom spectrophotometer. The total polyphenol content was expressed as gallic acid equivalent (GAE)/100 g of the sample. For this step, a calibration curve was obtained with different concentrations of GA.

Table 2.1 The concentrations of the blend.

Sample	PLA (wt%)	Polyphenols (wt%)
PLA	100	0
PLA/DP1	99	1
PLA/DP5	95	5
PLA/DP10	90	10

Table 2.2 Viscosity and conductivity characteristics of the blends.

Sample	Viscosity (mPa s)	Conductivity (µs/cm)
PLA	250	16.57
PLA/DP1	195	16.87
PLA/DP5	190	17.47
PLA/DP10	184	21.9

2.2.4.2 Scanning electron microscopy

The morphology of the electrospun fibres of PLA/polyphenol blends was inspected by a field emission scanning electron microscope (FE-scanning electron microscopy

(SEM), Nova Nano SEM 650) at different magnifications. All specimens were sputter-coated with 2 nm gold before the use of the SEM.

2.2.4.3 Porosity

The porosity of the fabricated fibre mats was measured using the alcohol displacement method. The mats were immersed in 100% ethanol for 48 h until they were saturated and the percentage of porosity was calculated according to equation below:

$$P = (\mathrm{W2}-\mathrm{W1})/\rho \mathrm{V1} \times 100 \tag{1}$$

Here, W1 and W2 are the weight of the mat before and after immersion in ethanol respectively, V1 is the volume of the mat before immersion in ethanol and ρ is the density of ethanol, which equals to 789 kg/m^3.

2.2.4.4 Contact angle measurements

The hydrophilicity of the electrospun fibre mats was assessed by determining contact angles. Contact angle measurements were carried out with water using a DataPhysics contact angle system OCA 20, in order to determine the changes in the hydrophilic character of pure PLA and its blend with different concentrations of polyphenol. Sample thicknesses of the fibre mats were 0.43 mm. All measurements were carried out with water, and each sample was measured with a minimum of five drops.

2.2.4.5 Tensile testing

Samples were cut into 1-cm width and 10-cm length rectangular pieces and mechanical testing was conducted at room temperature using LF LLOYD INSTRUMENTS (An Ameter Company) at a crosshead speed of 5 mm/min. Three specimens were used for each sample and the reported values are the average of obtained results.

2.2.4.6 Dynamic mechanical analysis

Effect of polyphenol loadings on the glass transition temperature (Tg) of PLA was studied using dynamic mechanical analyzer (DMA) (RSA-G2, TA Instruments, USA) in tensile mode. Rectangular specimens with dimensions of 15 mm × 3 mm × 0.05 mm were cut from the samples. Change in tan δ and storage modulus vs. temperature was determined from the constant value of the elastic modulus on 0.1–200 μm displacement and 30–100 °C temperature ranges at a heating rate of 5 °C/min and a frequency of 1 Hz. The specimens were subjected to a tensile deformation of 0.1%.

2.2.4.7 Cell line and culture conditions

NIH/3T3 fibroblast cell line was obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured using Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS and 0.1% penicillin/streptomycin, inside a humidified CO2 incubator (Thermo Fisher Scientific, USA) at 5% CO2 and 37 °C temperature. For biological assays, cells were cultured in either 6-well plates or 24-well plates until confluence. All the experiments were performed using cells with passages ranging from 15 to 20. Initial concentrations of 1 × 105 cells/well for 6-well plates and 5 × 104 cells/well for 24-well plates were seeded to each well in the experiments. Cells were then incubated for 24 h in CO2 incubator until they reach full confluence at which point tested scaffolds were placed into cell culture wells. Scaffolds were sterilized by UV irradiation (20 min) prior to placement into wells containing cells. Cells were cultured in the presence of scaffolds for an additional 24 h. 3.8 Cell proliferation assay Cell proliferation was quantified with 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl-tetrazolium bromide known as MTT assay (Sigma, USA). This test indicates the number of viable cells and the level of metabolic activity of the cells in the presence of scaffolds [18]. For MTT assays, we cultured cells in 24-well plates. After incubation period with scaffolds (24 h in CO2 incubator), the medium and scaffolds were discarded. The cells were then rinsed in PBS twice. We then added 50 μL of MTT solution (5 mg/mL in PBS) to each well and incubated for 4 h. After the incubation period, the reaction was stopped by discarding solution (MTT and DMEM medium) and the converted dye was dissolved in dimethyl sulfoxide (DMSO) for 15 min. Of the purple solution, 100 μL was taken from each sample, transferred to a 96-well plate and subjected to optical density (OD) measurements of the converted dye, at a wavelength of 570 nm according to the manufacturer’s instructions, using a EPOCH2 microplate reader (BioTek). To quantify the effect of scaffolds on cell proliferation, Eq. 2 was used as follows:

$$\text{Cell proliferation}\,(\%) = (\text{OD of sample}/\text{OD of control}) \times 100 \qquad (2)$$

All the experiments were repeated 3 times and absorbance was measured in triplicates.

2.2.4.8 Live/dead assay

A live/dead cell viability/cytotoxicity kit (Molecular Probes, USA) was used to visually evaluate the effect of scaffolds on cell viability. The live/dead stain was prepared by adding 5 µL of ethidium homodimer-1 (EthD-1) and 5 µL calcein AM to 10 mL serum-free medium (final concentrations of 1 µM EthD-1 and 2 µM calcein). Live cells convert calcein AM into green fluorescent calcein staining whole cytoplasm while EthD-1 penetrates through damaged membranes of dead cells and upon binding to nucleic acids produces a bright red fluorescence of the nucleus [19]. For live/dead assay, we used 6-well plates. After incubation period with scaffolds (24 h in CO_2 incubator), the medium and scaffolds were discarded. The cells were rinsed in PBS twice and then were treated with prepared stains for 1 h. Finally, the cells were visualized using an inverted fluorescence microscope at × 40 magnification (OLYMPUS IX-71, UK) and images were captured by Zen imaging software using an Axiocam camera (Zeiss). From the obtained images, cell viability was calculated using Eq. 3 below:

$$\text{Viability}\,(\%) = L/(L + D) \times 100 \qquad (3)$$

Here, L and D are the total number of visualized living and dead cells, respectively. Experiments were performed in triplicate for each experimental scaffold run.

2.2.4.9 Cell migration assay

Effect of PLA/polyphenol scaffolds on cell migration was determined by the scratch cell migration assay. The scratch was created in confluent cells on 6-well plate using a pipette tip. The cells were then rinsed with PBS to remove any freefloating cells and debris. Serum-free cell medium was then added, and culture plates were incubated at 37 °C in the presence of scaffolds for 24 h. Images of scratched areas were captured with an inverted microscope (Olympus IX-71, UK). Experiments were performed in duplicates for each scaffold.

2.3 Results and discussion

2.3.1 Total phenolic content

The total phenolic compounds in date palm fruit are expressed as milligrammes of gallic acid equivalent in 100 g of fresh weight of the date fruit. The equation obtained from constructed plot of standard gallic acid was used to calculate the total phenolic content (line A = mx + C, R2 = 0.98). Here, A is the absorbance and C is the concentration as gallic acid equivalent (mg/mL). We found 2.5 mg GAE/100 g of fresh weight, similar to the result reported for Algerian and Iranian date palm fruits [20], where it ranged from 2.49 to 8.36 mg GAE/100 g of dry weight. The variation in total phenolic content is most likely due to factors such as maturity of the fruit and geographic origin.

2.3.2 Morphology and hydrophilicity

The surface morphologies of the electrospun fibres are shown in Figure 2.2. It can be observed that continuous fibres were obtained successfully in both pure PLA and PLA blended systems. It is clear that the fibre diameter is affected by the addition of the polyphenol: as the polyphenol contents increases, the fibre diameter decreases. This is an expected finding since high solution viscosity of pure PLA has been associated with the production of larger fibre diameter [21]. Our observation is consistent with a recent study by Yanna et al. [22] who also found that addition of polyphenol extracted from tea resulted in decreasing the fibre diameter due to viscosity lowering. Related contact angles of fibre mats are also shown in Figure 2.2, evaluated by Image J software. Both fibre diameter and contact angle decrease with increasing polyphenol content; while for pure PLA, the average fibre dimeter is 1.8 μm and the contact angle is 140°, for PLA/DP10, the average fibre dimeter is 500 nm and the contact angle is 98°. These result show that the blend hydrophilicity increases and fibre diameter decreases with the increase in polyphenol concentration in the PLA.

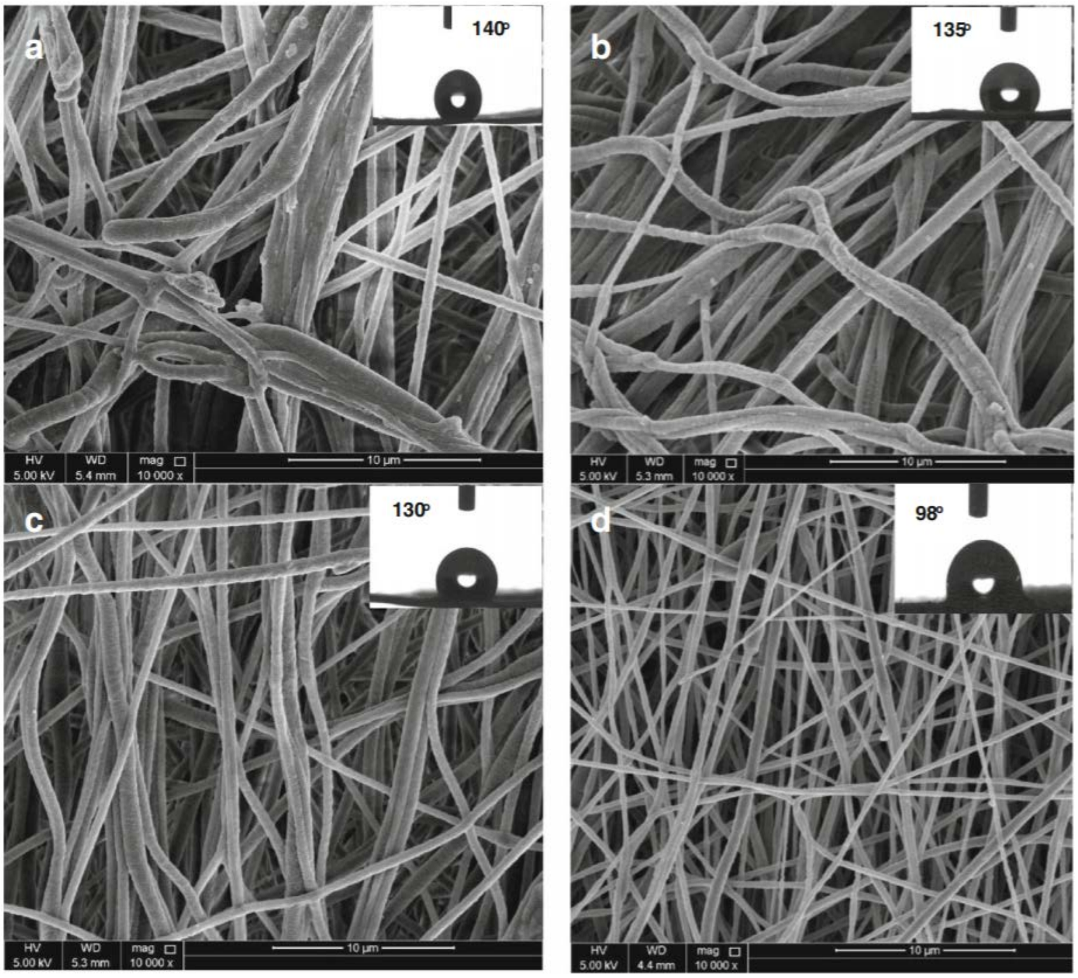


Fig. 2.2 Electrospun fibres: (a) pure PLA, (b) PLA/DP1, (c) PLA/DP5 and (d) PLA/DP10 with their corresponding contact angles.

2.3.3 Porosity

The porosity of the fabricated PLA/DP scaffolds was evaluated using the alcohol displacement method. As shown in Table 3, addition of polyphenol decreased the porosity. While pure PLA scaffold has 90% porosity, PLA/DP10 scaffold had 82% porosity. Same observation reported by Yaru Wang et al [11]

Table 2.3 Porosity percentages of PLA compared with blended systems.

Sample	Porosity (%)
PLA	90
PLA/DP1	88
PLA/DP5	85
PLA/DP10	82

2.3.4 Mechanical properties

Mechanical characterization of electrospun nanofibres was performed by tensile testing measurements and dynamic mechanical measurements. Table 2.4 represents the tensile strength, the Young modulus of elasticity and the percentage of elongation at break for pure PLA and the PLA/polyphenol blend samples.

2.3.4.1 Tensile testing

Tensile strength and Young's moduli of PLA/DP nanofibres were significantly lower compared with pure PLA. As the polyphenol concentration increases, both tensile strength and Young's Modulus decreased. The presence of polyphenol also resulted in lower elongations. The elongation at break of the blended systems was significantly lower than that of the pure PLA. The decreases in tensile strength resulted from the weakening or plasticization of the PLA molecular chain bonding which could explain the significant drop in the elongation at break values in the blends [23].

Table 4.4 Tensile testing results of neat PLA compared with different concentrations of polyphenols.

Sample	Tensile strength (MPa)	Youngs modulus (MPa)	Elongation at Break (%)
PLA	10.5	632.3	11.6
PLA/DP1	7.8	233.1	1.1
PLA/DP5	3.1	188.2	0.5
PLA/DP10	1.5	61.9	0.01

2.3.4.2 Dynamic mechanical analysis

The dynamic mechanical behaviour of the electrospun mats containing PLA/DP was measured in the broad temperature range as shown in Figure 2.3. The damping properties of the material give the balance between the elastic and viscous phases in a polymeric structure [24]. Figure 2.3 shows an increase in the T_g peak broadening with addition of polyphenol. The T_g for the neat PLA was 67 °C and did not change with polyphenol addition. The T_g broadening and intensity decrease could indicate some interactions between the hydroxyl groups of the polyphenol and the carbonyl groups of the polylactic acid. The dynamic storage modulus (E) is defined as the stress in phase with the strain in a sinusoidal shearing deformation divided by the strain [25]. The variation in the storage modulus as a function of temperature for the studied blends is illustrated in Figure 2.4. As the temperature increases, the plateau region drops rapidly due to the increase in molecular mobility of the polymer chains at the T_g, indicating that the scaffold become more plasticized having easier mobility [26].

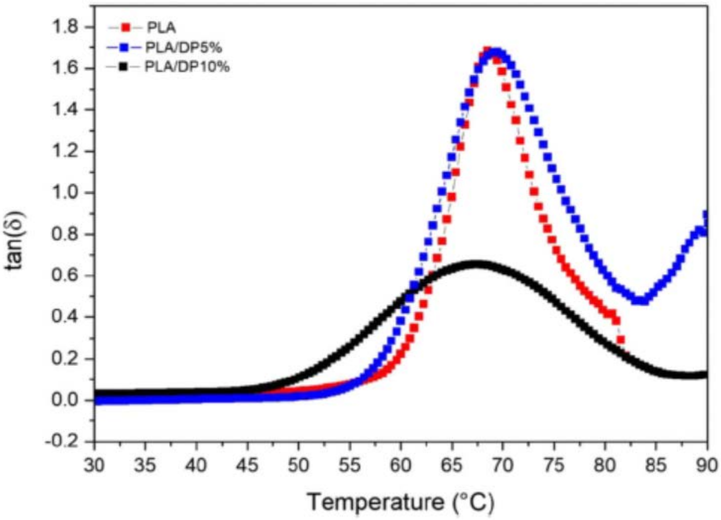


Fig. 2.3 Tan δ vs. temperature for pure PLA and its blended systems.

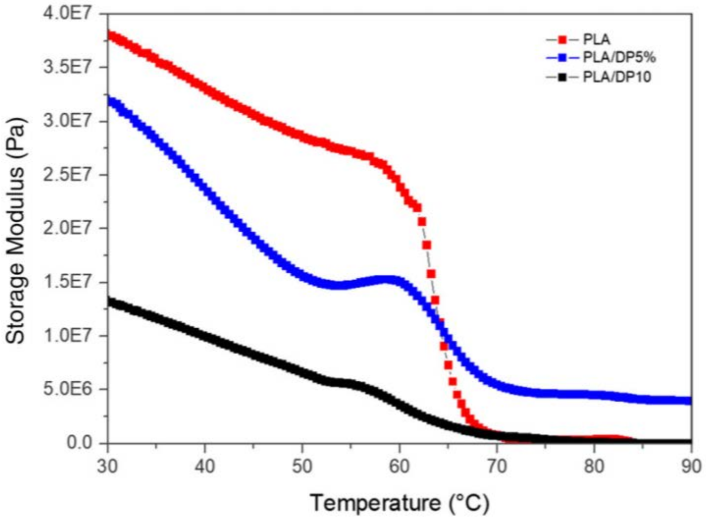


Fig. 2.4 Storage modulus vs. temperature of neat PLA and its blended systems.

2.3.5 Cell proliferation

As a strategy to determine the cytocompatibility of PLA and PLA/DP scaffolds, we performed cell proliferation studies using 3T3 fibroblast cell line as explained in Figure 2.5. MTT assay was used for this purpose and the result is shown in Figure 2.6. As expected, the proliferation of cells grown in the presence of PLA scaffold was comparable with those grown on bare cell culture plates. After 24 h of cell culture, the viability of cells cultured with pure PLA membranes was 100.2 ± 4.9%. Proliferation of cells grown in the presence of PLA/DP5 scaffolds was 115.4 ± 1.4%. Cell proliferation was much higher for cells cultured with PLA/DP10 scaffolds (124.0 ± 1.7). Scaffolds loaded with DP10 showed the highest set of viability results compared with the bare PLA and controls ($P \leq 0.05$) investigated in this study.

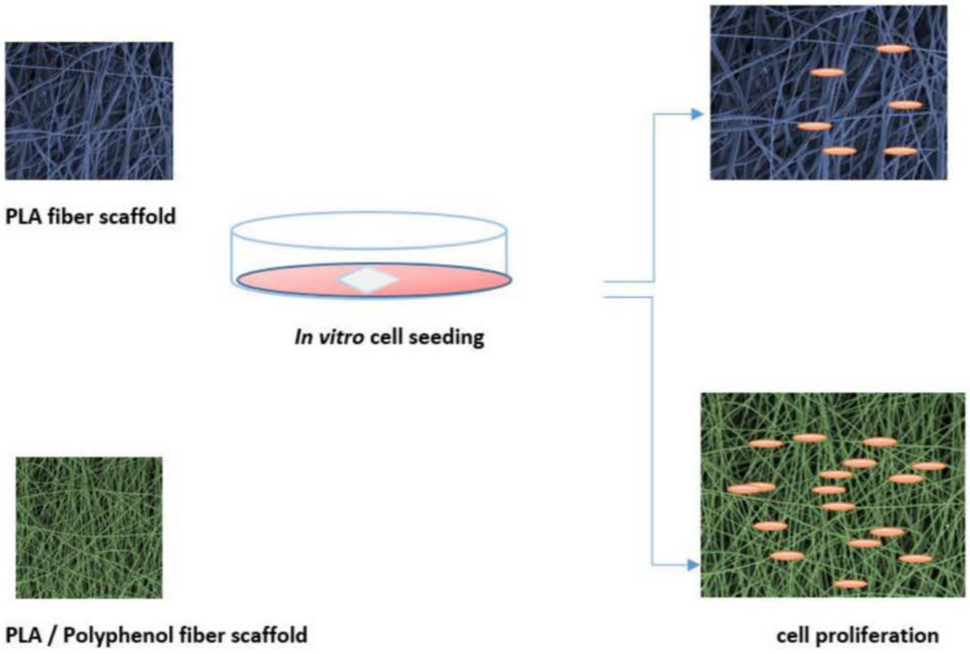


Fig. 2.5 Schematic illustration of the mechanism of cell proliferation on PLA/polyphenol nanofibre scaffolds.

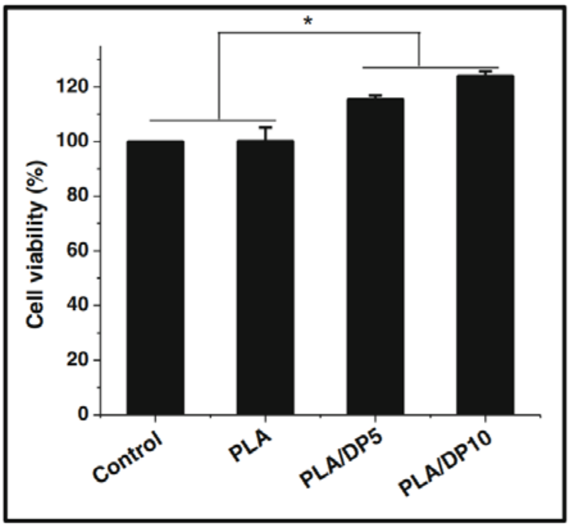


Fig. 2.6 Proliferation of 3T3 fibroblast cells in the presence of PLA and PLA/DP scaffolds.

2.3.6 Cell viability

Direct visualization of viable cells is important in TE to evaluate the effect of scaffold conditions on cell behaviour. Cultured fibroblasts were cultured in the presence of neat PLA or PLA/polyphenol scaffolds and then were stained with a live/dead assay, where live cells fluoresce green and dead cells fluoresce red. As shown in Figure 2.7, both the control and PLA scaffold-treated groups showed relatively similar number of live and dead cells. The percentage of green fluorescent (live cells) was higher in PLA/DP5 scaffold treatment groups compared with the PLA scaffold groups. Very interestingly, in the PLA/DP10-treated cells, most cells were green, indicating the highest set of viability and cell proliferation. These results suggest that the DP incorporation in PLA scaffolds helped to improve the cell viability and cell proliferation of seeded 3T3 fibroblasts. However, we did not observe considerable variation in relative ratio of live and dead cells, which indicate the nontoxicity of all the tested scaffolds irrespective of DP content.

In support to our results, earlier studies demonstrated that PLA-based scaffolds do not elicit any cytotoxic effects on human foetal osteoblasts cells [27]. Another interesting study indicated that PLA nanofibre scaffolds could improve the mineralization rate of stem cells isolated from human exfoliated deciduous teeth [28]. The increased cell viability and proliferation upon treatment with electrospun PLA/DP scaffolds might be because of the improved hydrophilicity of the scaffolds due to the presence of polyphenol. Increased hydrophilicity in cell culture scaffolds is expected to enhance cell proliferation as shown in previous studies from our group [29, 30]. It is known that cells prefer to grow on moderately hydrophilic surfaces, since it enables the polymer to adsorb cell adhesion proteins [31, 32]. In addition to that, the released polyphenol from the scaffolds might have played an active role in improving cell viability and proliferation. Earlier reports suggest that antioxidant property of polyphenol can minimize ROS-induced cell damage and improve cell viability [33]. In summary, the present study provides a proof of concept for the ability of polyphenol to enhance cell viability and proliferation of fibroblast cells, which will have potential applications in tissue engineering and wound healing.

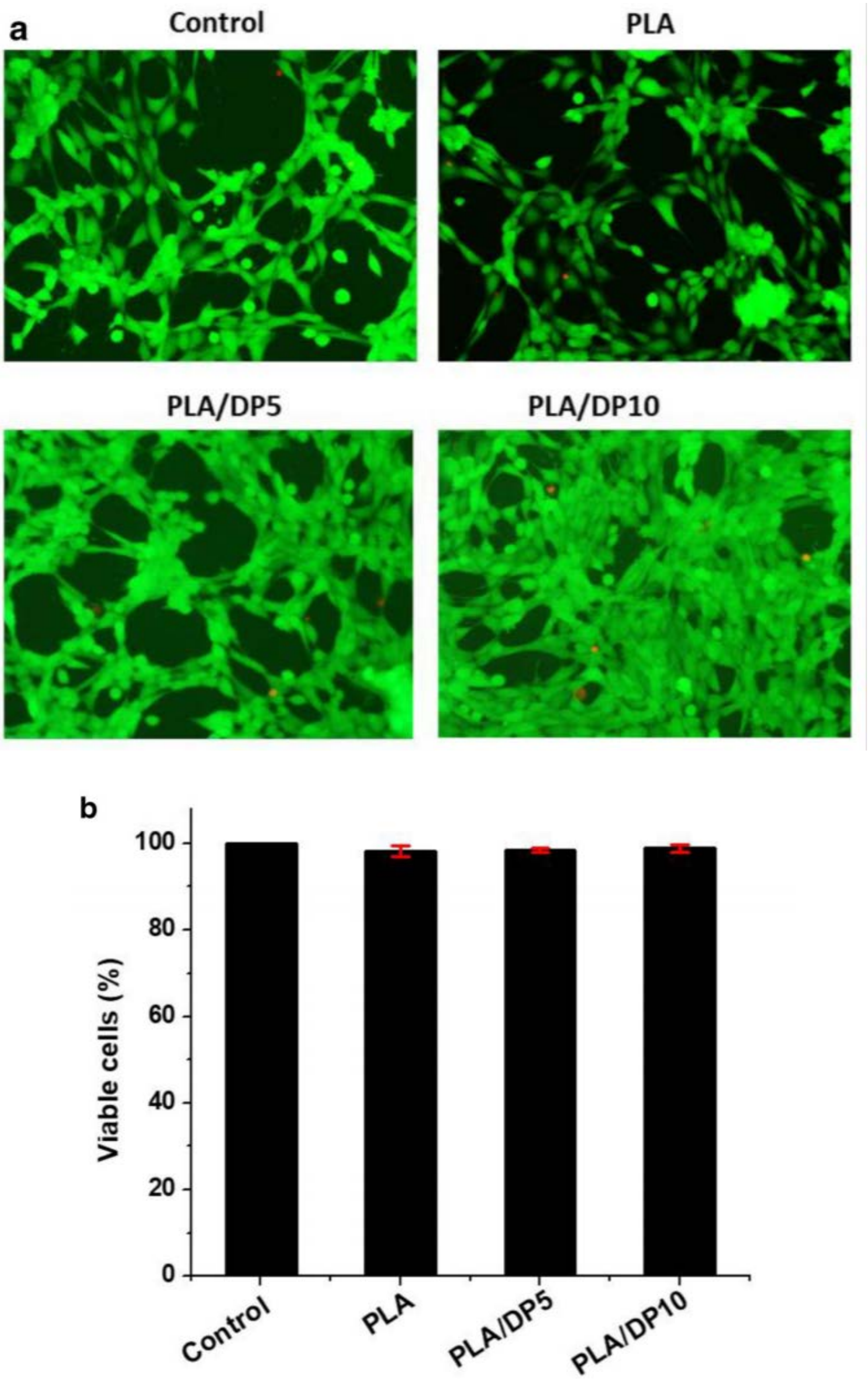


Fig. 2.7 Live/dead assay on NIH/ 3T3 cells treated with PLA, PLA/ DP5 and PLA/DP10 (a). Quantification of viable cells from live/dead assay (b).

2.3.7 Cell migration

The wound healing scratch test can be used to understand the effect of wound coverage materials to accelerate or decelerate the healing of wounds in vitro [34]. The scratch assay results obtained using the scratch test are given in Figure 2.8. After 24 h of treatment with the scaffold, about 20% of scratched area was healed in control due to the migration of 3T3 cells into the scratched area. Relatively similar trend was observed on the PLA membranes. In contrast, both PLA/DP5 and PLA/DP10 scaffold groups considerably enhanced the scratch healing compared with the pure PLA scaffolds and control groups. Overall, it has been observed that polyphenol-containing PLA scaffolds improved the cell migration for the in vitro wound model.

It has been previously observed that tea polyphenols loaded in chitosan scaffolds improved in vivo wound healing [35]. Further, biomolecules from date palm might also have played some role in wound healing in a similar manner [36]. In wounds, over-expression of proinflammatory cytokines might affect cellular functions and would retard the natural process of cell migration and wound healing [37]. Developed scaffolds incorporated with antioxidant DP could minimize the oxidative stress and subsequently promote healing process. However, detailed in vivo studies need to be performed to fully verify the wound healing potential of the developed scaffolds in animal models. Our future investigations will focus on such in vivo wound healing studies and the histological analysis of excised skin from the healed wound area

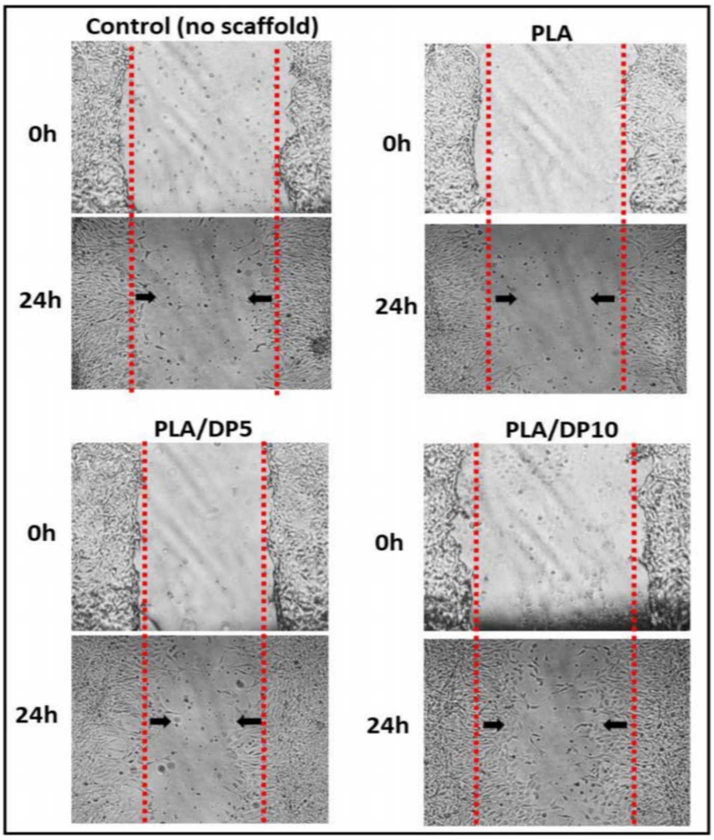


Fig. 2.8 In vitro wound healing assay using 3T3 cells. Microscopic images showing the scratched area at the beginning of experiment (0 h) and after 24

CHAPTER 3

This chapter will be submitted as a paper for publication.

In this part the morphology, thermal stability and mechanical properties of melt-mixed poly(lactic acid) (PLA)/polyphenol blends were investigated. Different concentrations of polyphenol extracted from two different plant sources were mixed into PLA at concentrations of 1, 5 and 10 wt.%. The blends were prepared through mixing in a Brabender Plastograph internal mixer. The oxidative thermal stability (OIT) of the different samples was studied using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Both the investigated polyphenols improved the thermal stability of PLA, but green tea polyphenols improved the thermal stability of PLA more than date palm polyphenol extract due to a wider range of polyphenols in green tea than in date palm fruit contains. The Young's modulus and tensile strength significantly reduced with increasing polyphenol content, the glass transition decreased and the elongation at break increased, indicating enhancement in the ductility as shown from SEM figures. This work investigate the influence of polyphenols as thermal stabilizer and plasticizer within PLA matrix.

Table 1 Main constituents in date palm fruit and green tea leaves.

Constituent	**Date Palm Fruit [% w/w]**	**Green Tea Leaves [% w/w]**
Ash	1-8	2-8
Lipids	0.12-0.47	NA
Proteins	1.7-4.7	NA
Catechins	1.74–3.39 mg GAE/100g	65-85 %
Total sugar	71-80	1-3
Total polyphenol content	3.1- 30 mg GAE/100 gm	90-330 mg CAE/100g

Table 2 Main minerals obtained by ICP for both additives

Constituent	**Date Palm Fruit (ppm)**	**Green Tea Leaves (ppm)**
Ca	78.7	38973.5
K	1163.8	1015.5
Mg	18.5	662.5
Mn	1.55	36.5

1. **Materials and methods**

2.1 Materials

The PLA used in this study is a high molar mass biopolymer (Ingeo™ Biopolymer 2003D), obtained from NatureWorks, LLC (USA). It is transparent with a density of 1.24 g cm^{-3}, melt flow index of 6.0 g/10 min (2.16 kg/210 °C). The extraction of polyphenols from date palm fruit was described in a previously published paper [3]. A commercial polyphenol, extracted from green tea leaves, was obtained from Natures aid UK.

2.2 Sample preparation

The samples were prepared by melt-mixing using a Brabender Plastograph internal twin-screw mixer. The added contents of the polyphenols from both the date palm fruit and the green tea leaves were 1, 5 and 10 wt.%. The PLA was added into the Brabender mixer and 'mixed' for 5 min. at 180 °C and 30 rpm screw speed before the polyphenols were added. The mixing then continued for another 10 min. The mixed samples were compression molded into 0.13 mm thick at 180 °C for 5 minutes using a hydraulic press at a pressure of 1 ton.

Table 2 Sample codes used in this work

Sample code	PLA (wt.%)	Polyphenol (wt.%)	Polyphenol source
PLA	100	0	
DP1	99	1	Date palm fruit
DP5	95	5	
DP10	90	10	
GP1	99	1	Green tea leaves
GP5	95	5	
GP10	90	10	

2.3 Sample analysis

HPLC characterization of the extracts was performed in an Agilent (HP 1090) liquid chromatograph equipped with a C18 reverse phase Gemini column with an internal diameter of 4.6 mm and a length of 250 mm. The mobile phase comprised of two solvents, 2% acetic acid in deionized water (solvent A) and acetonitrile (solvent B). The flow rate of the mobile phase was set to 1 mL min^{-1}. the gradient profile of the mobile phase was gradually changed by varying the residence time and percentage of solvent A from 10 min of 95% solvent A + 5% solvent B, to 1 min of 90% solvent A + 10% solvent B, to 9 min of 85% solvent A + 15% solvent B, to 10 min of 60% solvent A + 40% solvent B, to 10 min of 40% solvent A + 60% solvent B, and finally to 5 min of 100% solvent B. Eluent detection was carried out using a diode-array UV detector, where measurements were made at wavelengths, 278 nm. Solutions using the concentrated extracts (date palm or green tea) were prepared by dissolving 2 mg of the extract in a 10 mL of 2% acetic acid in methanol solvent system. The injection volume was 10 µL from the above-mentioned solution.

The fracture surface morphology of the PLA blends was examined by a field emission scanning electron microscope (Nova Nano-SEM 450 from FEI, Czech Republic. The liquid nitrogen fractured surfaces of the specimens were covered by 10 nm thick gold layers.

The effect of polyphenol loadings on the glass transition temperature (T_g) of PLA was studied using dynamic mechanical analysis (DMA) (RSA-G2, TA Instruments, USA) in tensile mode under an initial strain of 0.03% at a frequency of 1 Hz, and heating from room temperature to 100 °C.

The tensile properties were determined in a universal testing machine Instron Model 5567 (Bucks, UK) at a crosshead speed of 10 mm min^{-1} according to ASTM D638.

The DSC analyses were performed in a Perkin Elmer DSC8500 differential scanning calorimeter. The sample masses were 6 ± 1 mg and the analyses were done in a nitrogen atmosphere (flow rate 60 ml min^{-1}). The samples were heated from 0 to 200 °C at 10 °C min^{-1}, cooled to 0 °C at the same rate, and re-heated to 200 °C at the same rate.

The oxidative thermal stability of the samples was investigated by determining the oxidation induction time (OIT) in the DSC. Open aluminium pans, containing 5 ± 1 mg sample, were used in the analyses. The samples were heated under nitrogen flow

(60 ml min^{-1}) at a rate of 10°C min^{-1}, from room temperature to 220 °C, and after 6 min of isothermal at this temperature, the purge gas was switched to oxygen. The heat flow was recorded under these isothermal conditions as function of time. The OIT was determined from the onset of the oxidation peak, which formed after a certain period. The thermal decomposition of the samples was evaluated by using a Perkin Elmer TGA 4000 thermogravimetric analyzer. The analyses were carried out on 5-10 mg samples at 10 °C min^{-1} from 30 to 600 °C. Tests were conducted in both inert (nitrogen, 60 ml min^{-1}) and oxidative (oxygen, 60 ml min^{-1}) atmospheres.

2.4 Characterizations of the additives

2.4.1 Thermogravimetric analysis (TGA)

The thermal degradation of the green tea and date palm fruit extracts were evaluated by TGA under nitrogen are shown in Figure 1. It is clearly observed that the commercial green tea polyphenols has a higher thermal stability than the date palm extract , probably due to the existence of tannins at retention time 2.3 min as confirmed by HPLC [63]. Thermal degradation of tannin is around 350^o C this also reported by zhyux [64]. four main decompositions steps were observed in green tea thermogram nearly as following: 200, 250, 350 ^{o}C , this belong to degradation of tannins in to [65 ,66] , however the observed decomposition steps of date palm were earlier, this attributed to the existence of glucose and total sugar in date palm fruit compared with green tea that accelerate the decomposition. The residues of both additives around 30 % in weight attributed to the composition of organic as shown in table1.

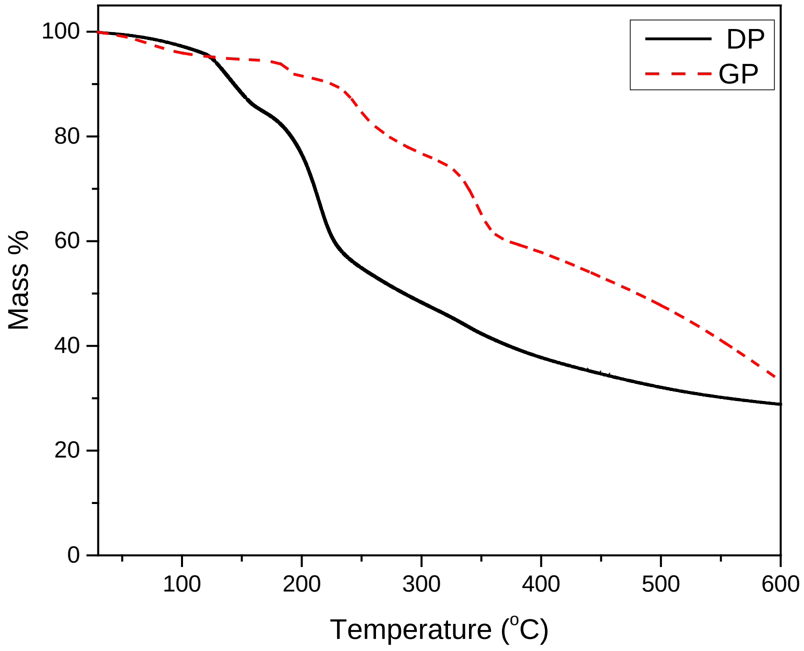


Figure 1 TGA curves for polyphenols extracted from date palm fruit (DP) and the commercial green tea (GP) polyphenols under nitrogen.

2.4.2 High-pressure liquid chromatography (HPLC)

Figure 3 shows the HPLC chromatograms of the DP and GP polyphenols. These chromatograms and [67,68] were used to determine the main chemical composition of each of the two types of polyphenols (Table 2). It is clear from this table that there is a significant difference between the chemical compositions of the DP and GP polyphenols, of which the most important is the presence of cinnamic acid in GP.

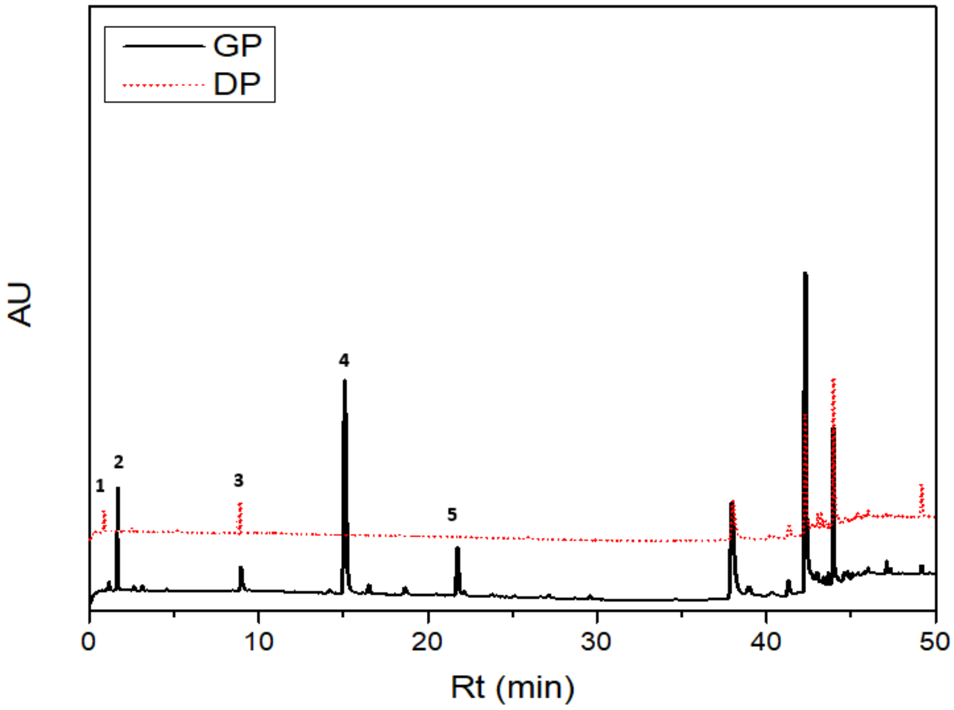


Figure 3 HPLC chromatogram of date palm polyphenol extract (DP) and green tea (GP) polyphenols at wavelength 278 nm.

	Phenolic compound	R.t. (min)
1	Gallic acid	1.2
2	Tannic acid	2.3
3	catechin	8.5
4	Epicatechin	15
5	Cinnamic acid derivative	21

2. Results and discussion

3.1 Morphology

The SEM photos at different magnifications of the tensile fractured surfaces of neat PLA and the 10 wt.% polyphenol containing samples are shown in Figure 4. It shows that the neat PLA has a rigid morphology and fractures without elongated fibrils. The DP10 and GP10 samples show clear ductile deformations. This is especially obvious in Figure 1(a,d,g). If one compares Figure 4(d) with Figure 4(g), it is clear that the polyphenols extracted from the green tea leaves has a stronger influence on the PLA ductility than the polyphenols extracted from the date palm fruit.

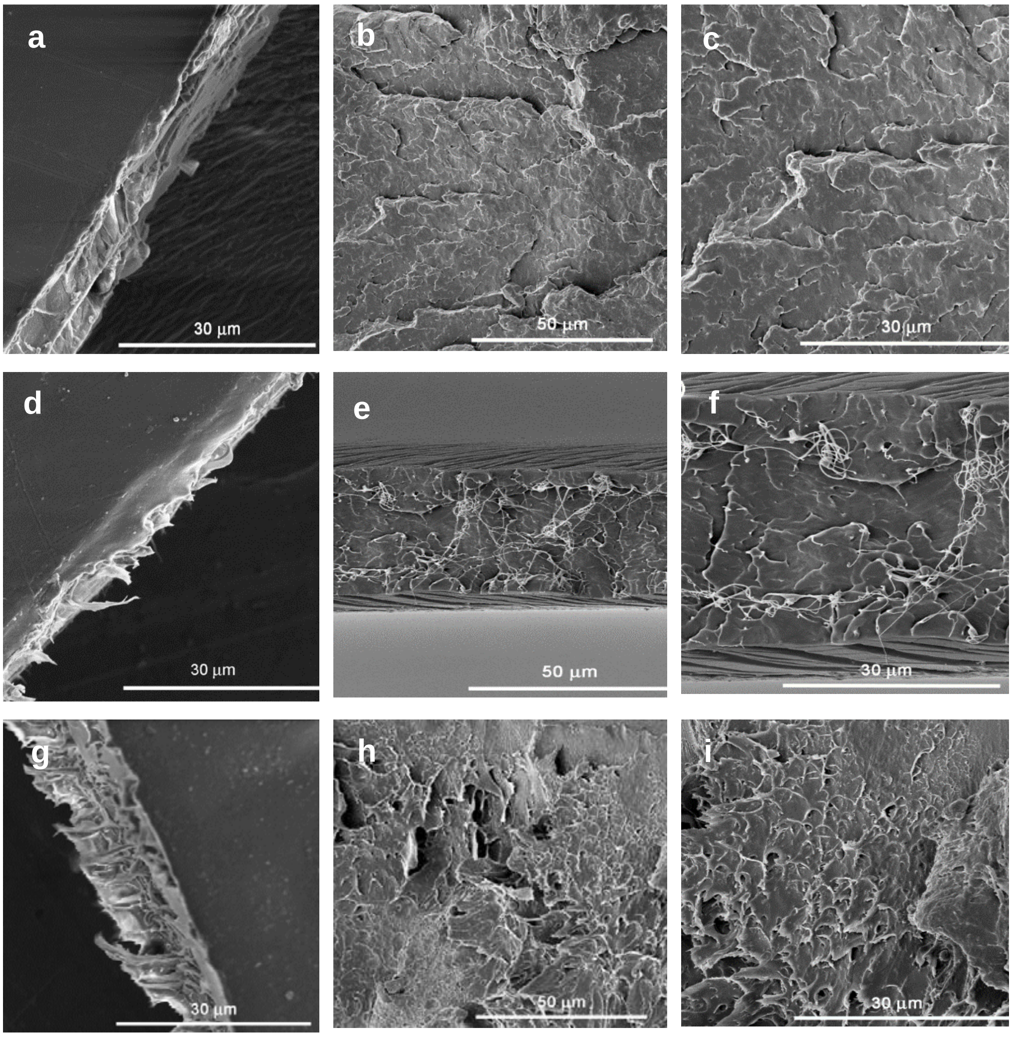


Figure 4 SEM images of fractured surfaces of (a-c) neat PLA, (d-f) DP10, and (g-i) GP10 at different magnifications.

3.2 Mechanical properties

3.2.1 Tensile testing

Table 3 summarizes the tensile properties of the different investigated samples. For both types of polyphenols, the blends show a decrease in Young's modulus and tensile strength and an increase in elongation at break, with increasing polyphenol content in the blends. This is to be expected, because the polyphenols clearly act as plasticizers [69] that increase the free volume in the PLA samples [6], giving rise to more ductile samples (see SEM results discussed in section 3.1)

Table 3 Tensile properties of neat PLA and polyphenol containing PLA blends.

Sample	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
PLA	26.3	1278	0.25
DP1	20.1	1165	0.58
DP5	5.6	1079	0.94
DP10	4.8	913	1.50
GP1	11.6	1009	0.80
GP5	11.2	954	0.98
GP10	5.5	497	1.86

Another interesting observation is that the Young's modulus decreased and the elongation at break increased more significantly for the samples containing the green tea leaves extracted polyphenols (GP). This is in line with the higher ductility observed for these samples (see discussion in section 3.1). Daniele et al

Chavez et al. [70] studied the effect of extruded poly(lactic acid) with pecan nutshell bio-composites as source of polyphenol, and found that the tensile strength, tensile modulus and elongation at break gradually reduced with increasing polyphenol content. He concluded that the mechanical properties of the composites were influenced by the additive compatibility with the matrix. This is in line with our own observations and the explanation is probably the same.

3.2.2 Dynamic mechanical analysis (DMA)

Figure 5 shows the tan δ curves of all the investigated samples. The peak temperatures are summarized in Table 4. The glass transition temperature of neat PLA is around 65 °C, but the polyphenols in the PLA matrix led to decreases in the T_g for all the PLA/polyphenol blends.

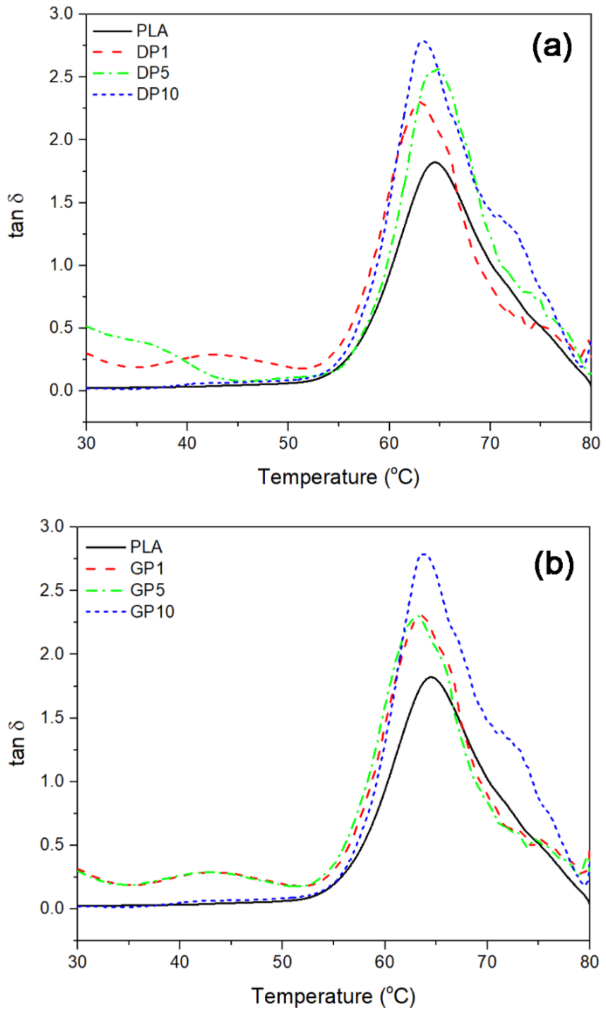


Figure 5 tan δ as a function of temperature for neat PLA and (a) the date palm fruit polyphenol containing PLA blends, and (b) the green tea leaves polyphenol containing PLA blends.

Gordobil et al. [71] studied the effect of Kraft lignin as source of polyphenol in PLA, and found that the addition of polyphenol enhanced the ductility, which was seen as a decrease in T_g. Their explanation is that the polyphenol, which generated greater free volume between the PLA chains, enhanced the chain mobility when a load was applied.

The decrease in T_g corresponds to the plasticizing effect of the polyphenol in the PLA matrix, while the increases in the intensities of the glass transition peaks for the blends correspond to the ratio of the dissipated energy to the energy stored per cycle of sample deformation at the glass transition temperature. This indicates that the neat PLA stored less energy compared to the blends, which explains the enhancement in the flexibility of the blends.

Table 4 Glass transition temperatures of neat PLA and the PLA/polyphenol blends.

Sample	DMA (T_g / °C)	DSC (T_g / °C)
PLA	65.2	55.8
DP1	64.2	50.8
DP5	62.5	50.3
DP10	61.3	46.7
GP1	63.4	51.9
GP5	65.1	51.3
GP10	64.2	51.1

3.3 Thermal properties

3.3.1 Differential scanning calorimetry (DSC)

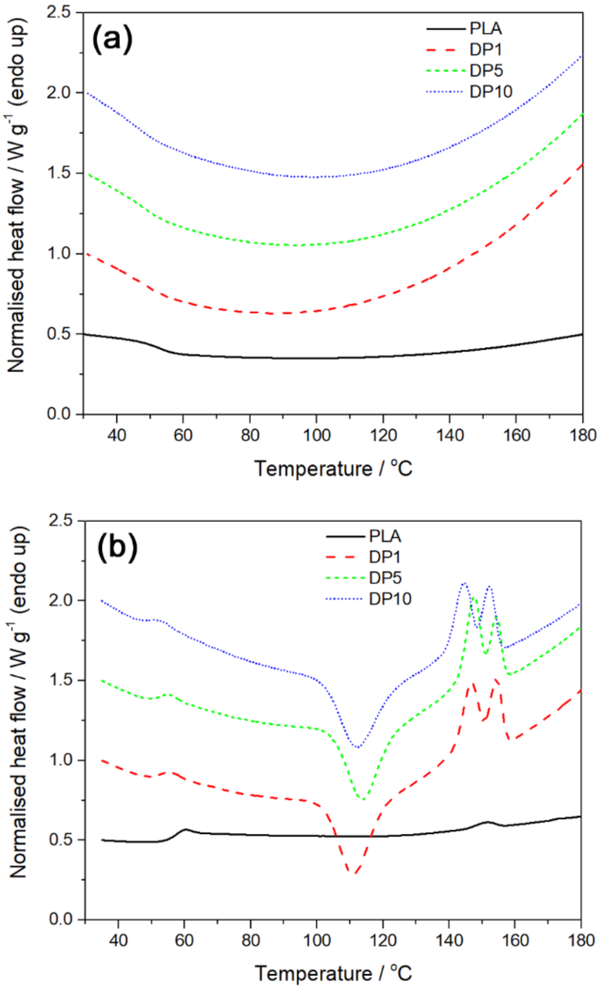
To investigate thermal behavior of PLA and the effect of polyphenols on the PLA matrix, DSC measurements were carried out. Thermal properties such as glass transition temperature (T_g), cold crystallization temperature (T_{cc}), cold crystallization enthalpy (ΔH_{cc}), melting temperature (T_m), and melting enthalpy (ΔH_m) are summarized in Table 5. The DSC cooling and re-heating curves are presented in Figure 6.

Table 5 Thermal properties of neat PLA and polyphenol blends.

	T_g (°C)	T_{cc} (°C)	ΔH_{cc} (J g^{-1})	T_m (°C)		ΔH_m (J g^{-1})
PLA	55.8	-	-	151.2		1.4
DP1	50.3	110.9	-25.7	146.8	154.5	28.3
DP5	50.7	111.6	-23.4	147.7	154.4	27.4
DP10	46.7	113.5	-22.0	144.7	152.2	27.9
GP1	51.9	112.9	-25.6	148.2	154.4	29.1
GP5	51.3	111.0	-26.6	142.6	150.0	26.5
GP10	51.1	99.0	-28.4	137.5	147.9	25.8

Figure 6 represents the cooling scans (a,c) and the second heating scans (b,d) of all the investigated samples. The neat PLA is amorphous as seen in Figure 3(a,c). The absence of crystallization peaks in these curve confirms the amorphous nature of the PLA.

A study done on PP [14] confirmed that the crystallinity of samples compounded with polyphenol extracted from wine waste decreased after the addition of the polyphenol. In our case, there was anyway no crystallinity in the PLA.



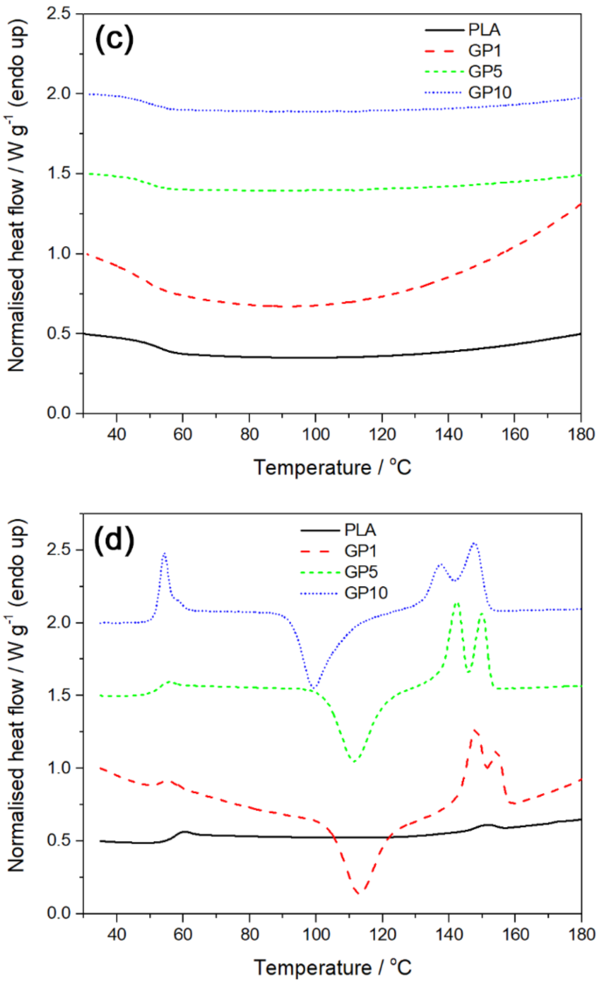


Figure 6 DSC cooling (a,c) and second heating (b,d) curves of PLA and the PLA/polyphenol blends.

The first endothermic transition is observed around 55 °C. This is due to the glass transition of the amorphous phase of the semicrystalline PLA [72] , the second endothermic peak observed at 151°C is associated with the melting of the PLA.

The same transitions are observed for the PLA/date palm polyphenols .it is observed gradual decreases in the glass transition temperature for the blends from 55 °C for PLA in to 46 °C for DP samples. This decreases attributed to increases in the free volume of the polymer chain, confirming plasticizing effect of date polyphenols.

From figure 6(b), it is clear no cold crystallization peak of the neat PLA; however, the sharp cold crystallization is clear for DP samples as well as GP samples, which means that the polymer chain become more mobile ,due to increases in the free volume. Moreover, it is observed a double melting peak of the blended systems this attributed to melting recrystallizing and melting of larger crystals effect and results of larger free volume forms from the polyphenols, same observation were seen with M. Jamshidian et al [73] . The enthalpy gained with PLA is less than blended system for date palm polyphenol, because the polyphenols as a filler is in liquid form increases the free volume, which lead to increases in the heat gained for melting.

On the other hand, the green tea polyphenol extract behaves differently compared with date polyphenols. The decreases in the cold crystallization peak attributed to better compatibility and interaction of polyphenols with polymer matrix due to the green tea polyphenols act as nucleating argent , which reduces the mobility of polymer chain compared with date palm polyphenols. More over the existence of tannic acid as main component in green tee structure, while the tannic acid is type of tannins with astringent like properties having phenolic acid component in its structure. It is considered that phenolic acid has higher affinity to lactic acid units of PLA, which enhances the compatibility of the blend [23].

The glass transition temperature were exhibit same behavior with date polyphenol, with decreases in the glass transition temperature confirming the plasticizing effect of polyphenols. The enthalpy needed to melt the polymer blend was observed to decreases from 1.4 J/g of PLA to 25 J/g observed in GP10.

Table 6 summarizes the oxidation induction time (OIT), which is commonly used as a measure of the oxidative thermal stability of a substance. A longer OIT indicates a higher oxidative stability of the sample. The table clearly shows that the addition of polyphenols enhanced the thermo-oxidative stability of the PLA, with the polyphenols extracted from green tea being more effective than those extracted from date palm fruit, giving significantly higher OIT values for the samples containing respectively 5 and 10 wt.% polyphenols in the PLA. Ambrogi et al. [75] also confirmed the effectiveness of the addition of bio-based compounds containing polyphenols as stabilizers and antioxidants for polypropylene (PP) on improving the thermo-oxidative stability of PP.

Table 6 OIT values for all the investigated samples.

Sample	**OIT (min)**
PLA	15
DP1	20
DP5	25
DP10	38
GP1	25
GP5	35
GP10	40

3.3.2 Thermogravimetric analysis (TGA)

Figure 7 shows the TGA curves of the different blends under nitrogen and oxygen atmospheres, respectively. The results from the TGA curves are summarized in Table 7.

All the samples degraded in a single step. If one compares Figures 7(a) and 7(b), as well as the values in Table 7, one can see that the GP polyphenols are much more effective in thermally stabilizing the PLA in nitrogen atmosphere. Only 1 wt.% GP increased the degradation temperature by about 60 °C. Larger contents of GP gave about the same results, which means that a significant thermal stabilization can be obtained with a relatively low GP filler content. Since GP were solid particles in the

PLA matrix, it is possible, if there was a strong interaction between the GP particles and the PLA matrix, that the GP particles immobilized the PLA free radical chains and degradation products, which would have led to these products evaporating only at much higher temperatures.

The DP polyphenols, on the other hand, caused a much lower increase in degradation temperature of only about 10 °C for DP1 and DP5 in nitrogen atmosphere, but for DP10 the increase in degradation temperature was about the same as that for the GP containing samples. It is therefore clear that there was much less interaction between the DP polyphenols and the PLA matrix.

As expected, the neat PLA started degrading at a lower temperature in an oxygen atmosphere (Figures 7(c) and 7(d)). Under these conditions, the thermal stabilization of PLA by the GP and DP polyphenols followed about the same trend as in the case of the nitrogen atmosphere, and the explanation for this behaviour is probably the same. The only difference is that in the case of the DP polyphenols, the onset of degradation increases almost linearly with increasing DP content in PLA (Table 7).

Similar observations were reported by Alessandro et al. [76] with addition of wine-waste as source of polyphenol derived additives. These were found to be very effective to improve the thermal stability of the polypropylene compared with neat polypropylene matrix.

Table 7 TGA onset of degradation temperatures ($T_{5\%}$), dTGA peak maximum temperatures, and mass residues at 600 °C of all the samples analyzed under nitrogen and oxygen atmospheres.

	Nitrogen			Oxygen		
	$T_{5\%}$ (°C)	dTGA peak (°C)	Wt.% residue at 600 °C	$T_{5\%}$ (°C)	dTGA peak (°C)	Wt.% residue at 600 °C
PLA	310.5	368.2	0.0	285.5	340.3	0.0
DP1	330.1	372.1	0.5	294.3	355.8	0.1
DP5	342.4	375.3	0.7	338.2	357.6	0.5
DP10	360.8	381.5	0.8	355.8	367.4	0.8
GP1	361.0	380.4	1.5	354.2	374.2	0.1
GP5	361.7	384.6	2.1	355.3	375.6	0.4
GP10	362.8	386.2	4.1	360.1	376.5	1.5

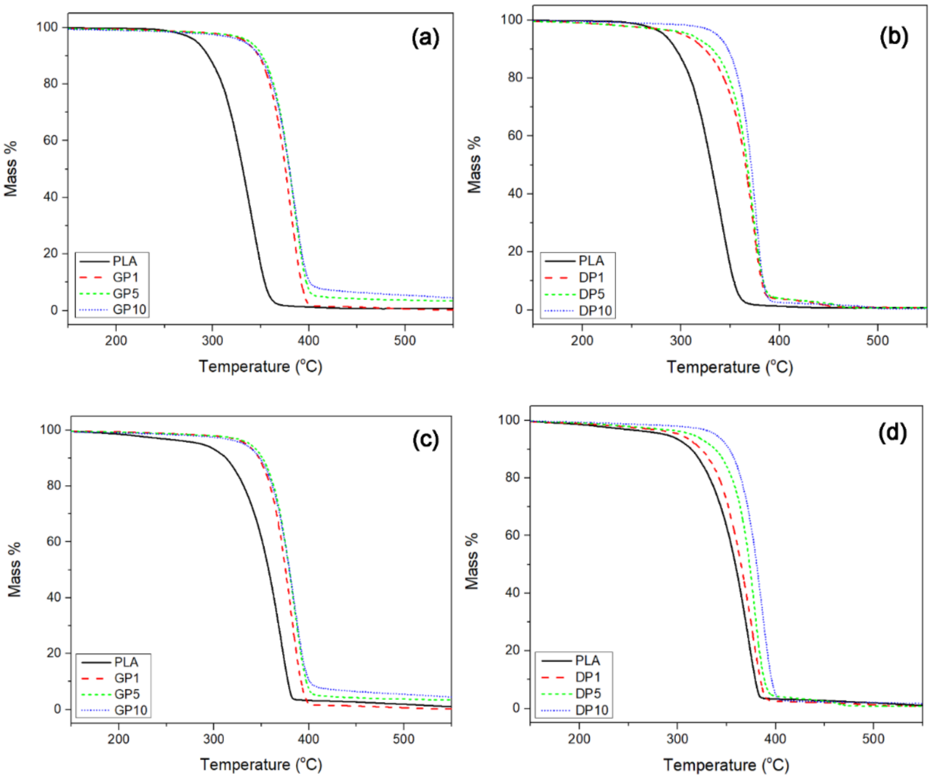


Figure 7 **TGA curves of all the investigated samples under (a,b) Nitrogen and (c,d) Oxygen atmospheres.**

General Conclusions

This thesis aimed to exploit the agro waste in to valuable product; however, one of the major issues of this century is the environmental pollution resulting from industrial activities such as plastic waste. Therefore using biodegradable polymer is essential to overcome on this problem. PLA is promising biopolymer to be used as suitable matrix for polyphenol extracted from expired date palm fruit.

The first part of the thesis aimed to demonstrate the potential benefits of incorporating polyphenol extracted from date palm fruit in PLA scaffolds for TE applications.

For this purpose, PLA scaffolds loaded with various concentrations of polyphenol were produced via electrospinning. Structural and mechanical characterizations were studied via SEM, contact angle, tensile testing and DMA. It was found that potential enhancement of cell culture characteristics of PLA/polyphenol scaffolds. In vitro cell culture studies using NIH/3T3 fibroblast cells showed that the scaffolds were biocompatible and supported cell adhesion, particularly when the content of the polyphenols increased. The enhanced cell adhesion was attributed to the increased hydrophilicity of the scaffolds with increasing the content of polyphenols. The surface tension of the scaffolds was assessed by analyzing the contact angle. The results showed that blend of PLA/DP scaffolds had higher hydrophilicities compared with hydrophobic PLA matrix. Thus, the enhancement in cell proliferation and cell viability and the increased cell migration through the scratch with higher loading of polyphenols were attributed to increased hydrophilicity. In summary, in this study, we demonstrated the potential utilization of PLA/polyphenol scaffolds for TE applications such as regeneration of damaged tissue or wound healing assays.

The second part of the study, PLA film was prepared in a Brabender Plastograph for industrial application. Two sets of samples containing two types of polyphenol extracted from different sources, green tea leaves and date palm fruit, were prepared for further analysis. The main objective of this study was to study the effect of the presence and amount of polyphenols on the thermal and mechanical properties of the PLA.

The presence of the polyphenol additives was found to be very effective in improving the thermal stability of the PLA, as could be seen from the TGA and OIT results. The mechanical properties were also affected by the addition of polyphenols, and it was found that the tensile modulus and tensile strength decreased significantly, while the elongation at break increased. This was confirmed by SEM observations. The glass transition temperature was determined by DMA and DSC, and in both cases it showed a decreasing value, confirming the plasticizing effect of the polyphenols. This effect caused the polyphenols to increase the amount of crystals formed during the cold crystallization of the PLA. All the polyphenol containing samples also shows a double melting peak as a result of the well-knownmelting-recrystallization-melting effect.

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