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ORIGINAL PAPER

Effect of lysozyme and glucose oxidase on the physical-mechanical and barrier properties of linear low-density polyethylene

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ABSTRACT

Biocomposites were synthesized from linear low-density polyethylene (LLDPE) with lysozyme, mixed lysosome, and glucose oxidase enzyme via a melt extrusion system. The aim of this work was to evaluate and characterize the effect of lysosome, mixed lysosome and glucose oxidase enzymes on the mechanical, water vapor transfer rate, and structural appearance of LLDPE biocomposite films. In this regard, 50 g of LLDPE pellets were applied along with 1%, 5%, and 10% (w/w) of lysozyme alone and lysosome mixed with glucose oxidase. Pure LLDPE was used as a control. Overall, the biocomposite with 5 and 10% (w/w) of mixed lysosome and glucose oxidase enzymes did not perform well and was not even sufficient for characterization. The surface structure of the biocomposites was examined through a digital microscope to identify the dispersion of enzymes within the LLDPE matrix, and it was found that at higher concentrations (10% w/w) a dense and large surface was formed. However, good dispersion and reinforcing ability of enzymes in the LLDPE matrix were observed at low concentrations of lysosomal enzymes (1 and 5% w/w). It was found that the mechanical strength and elongation-at-break of the biocomposite films increased at low enzyme concentrations (1 and 5 wt.%), but decreased with increasing enzyme concentration. On average, vapor permeability increased with increasing enzyme concentration. Besides, the Fourier transform infrared spectroscopy (FTIR) was used to determine the structural configuration of the enzymes in the LDPE matrix. Single sharp stretching breaks at 570 cm^{-1} (1, 5 and 10 wt.%) was corresponded to the existence of enzyme bands. Overall, the addition of lysosomes and glucose oxidase at low concentrations has great potential in the development of biocomposites compared to traditional plastic composites. **Polyolefins J (2025) 12: 53-60**

Keywords: Linear low-density polyethylene; lysosome and glucose oxidase enzymes; water vapor permeability; mechanical and barrier properties; FTIR spectroscopy.

INTRODUCTION

Packaging in the food industry is essential to maintaining the quality of products and ensuring their safety for consumers and helps control the shelf life of products. Packaging can solve many of the problems associated with currently used food preservation methods, and this has been proven by research in the last decade of this century [1,2]. Extending the shelf life of food products using active packaging does not require exposing food products to high temperatures, which leads to the destruction of heat-sensitive nutrients as traditional drying and pasteurization [3]. Creating active packaging also does not require a huge amount of energy or high costs, as is the case with the sublimation method [4]. Consumer safety will be guaranteed when using safe polymeric materials and natural antimicrobial additives in appropriate concentrations in the food industry. This cannot be guaranteed if traditional preservatives added directly to food are used [5]. There is also no need to release air from the packaging and destroy the structure of the food product, as is the case with vacuum packaging.

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When creating packaging materials with antimicrobial properties, the effect of additives on the properties and structure of the polymer matrix must be studied. Determining the ideal concentration of additives with antimicrobial properties depends largely on their effect on the properties of the polymer matrix. In addition to the antimicrobial properties that these additives can add to the polymer matrix, they can simultaneously lead to the deterioration of other properties such as physical, mechanical, and confinement properties. The physical and mechanical properties (failing stress and tension strain) of packaging play a crucial role in protecting the product from mechanical impacts. The confinement properties (permeability to water vapor) also affect the activity of microorganisms that cause food spoilage and thus the duration of food preservation [6,7]. Polyethylene belongs to the group of polyolefins and due to its properties, is one of the most widely used polymers for creating packaging materials. In addition to good manufacturability, polyethylene has low permeability to water vapor, so it can be used for the production of films, bottles, and bags. Polyethylene is an ideal choice for packaging a wide range of food products such as dry and liquid products, but is not suitable for packaging foods with high fat content due to its high oxygen permeability. There are many types of polyethylene, the most important of which are low-density polyethylene (0.910-0.940 g/cm^3), high-density polyethylene (0.940-0.970 g/cm^3) and linear polyethylene (0.916 - 0.940 g/cm^3) [8]. An extrusion plastometer or melt flow indexer is used to measure MFI, which is commonly stated in grams per 10 minutes (g/10 min) [9,10]. The melt flow index of the LLDPE, LDPE, with 0.8, 0.75 [11, 12], respectively and HPDE 3.95 g/10 min has been reported [13]. The most widely used type is low-density polyethylene due to its flexibility and ductility.

It is mostly used for packaging fresh and frozen meat, bread, and sweets. Linear polyethylene has higher physical and mechanical properties than low-density polyethylene, as well as higher heat resistance, therefore it is used to create transport packaging [14,15]. Linear low-density polyethylene has properties intermediate between low-density polyethylene and high-density polyethylene. Compared to low-density polyethylene, linear low-density polyethylene has higher mechanical and physical properties (elongation and strength) and high puncture resistance, as well as greater chemical resistance. When comparing two films of LDPE and linear LDPE with a thickness of 76 micrometers, we find that LDPE has a puncture resistance of (834 J/m^2), while the puncture resistance of linear LDPE is (1877 J/m^2). Also, when comparing linear low-density polyethylene with high-density polyethylene, we find that linear low-density polyethylene has high mechanical and physical properties (puncture resistance is 3 times higher) and chemical resistance. Linear low-density polyethylene has a higher melting point than high-density polyethylene, making it widely used for packaging hot foods. Thin bags can be made from linear low-density polyethylene and still have high strength. Linear low-density polyethylene retains its properties at low temperatures and is therefore widely used for packaging frozen foods. Linear low-density polyethylene has high barrier properties and is chemically inert, making it suitable for food packaging [16,17].

Lysozyme is an enzyme with antimicrobial activity that plays an immune role in the human body from birth. According to its chemical structure, it is a globular protein consisting of one polypeptide chain containing 129 amino acid residues in lysozyme, isolated from chicken egg white. Lysozyme is stable even over wide ranges of temperature and pH, stability is due to the presence of four disulfide bridges and due to the high isoelectric point "pI = 11". The enzyme reaches its maximum activity at pH 5. The lysozyme molecule has dimensions (4.5 × 3.0 × 3.0 nm) of an ellipsoidal shape [18,19].

Antimicrobial application of lysozyme enzyme in food packaging industry has been reported in the literature [20-22]. It has antimicrobial response against to bacteria and fungus cells and showed excellent potential for food preservative by replacing the chemical additives in the food packaging industry. The optimum heat sustainability of lysozome is between -18 to 100°C temperature. Lysozyme's use as an antibacterial agent in food processing has been documented in a number of studies [23-25].

Glucose oxidase is a glycoprotein with a molecular size of 150-180 kDa, consisting of flavin adenine dinucleotide. Glucose oxidase is widely used in the food industry, for example, to improve the properties of bread production - its freshness and consistency. Thanks to its antimicrobial properties, glucose oxidase is used as a preservative for meat [26].

The films based on polyethylene (PE) and ionomer film (ethylene/methacrylic acid copolymer containing 19% methacrylic acid) have been reported in literature [11]. A technology (Ugi reaction) was used for the immobilization of proteins (lysozyme and glucose oxidase) on polymer films (PA and ionomer film). First, the films were treated with hydrochloric acid for

20 min, after which they were washed with distilled water and dried in air. Then two solutions of proteins with different concentrations were prepared (glucose oxidase 160 μL: 24.8, 248, and 2480 units/mL and lysozyme: 40,000, 400,000, 4,000,000 units/mL). Two solutions were added to them (glutaraldehyde 40 μL and cyclohexyl isocyanide 16 μL). After this, a layer of protein mixture was formed on the surface of the films, and these films were incubated under certain conditions (4°C, 2-10 days). At the final stage, the film was washed with a potassium phosphate solution (0.1 mol/L, pH = 7) three times for 20 days. The results of Fourier transform infrared "FTIR" confirmed the presence of covalent bonds between lysozyme and glucose oxidase on the one hand and the surface of the films on the other hand [27].

There is currently limited study on the use of enzymes to enhance the mechanical, barrier, and antibacterial qualities of food packaging materials. Nevertheless, this investigation does not address the antimicrobial activities of the lysozyme and glucose oxidase enzymes. In this article, the effect of lysozyme and glucose oxidase on the physical, mechanical, and barrier properties of linear low-density polyethylene was studied and the changes occurring on the surfaces of the films obtained were elaborated using a digital microscope. The change in chemical properties of linear low-density polyethylene induced by enzymatic additions was evaluated by FTIR spectroscopy.

EXPERIMENTAL

Materials

Linear low-density polyethylene (100% LLDPE, melt flow index: 1 g/10 min at 2.16kg/190°C), (manufactured by SABIC, Saudi Arabia), lysozyme (manufacturer "Caglificio Clerici" Italy), and glucose oxidase (manufacturer "Trading House "Biopreparat"" Russian Federation) were used for the synthesis of the composite.

Sample preparation

Production of polymer films by extrusion with slit die

The biocomposite films were developed using the single screw extruder (model: ISD551M21B, INNO VERT, Moscow, Russia). The single screw speed in extruder is 90 rpm. Temperature modes for material processing by extrusion: 1 zone (T_1) = 110°C, 2 zone (T_2) = 120°C, 3 zone (T_3) = 130°C, 4 zone (extrusion head) (T_4) = 140°C.After all the parameters were acquired, the LLDPE, lysozyme, and glucose oxidase were added to the feed section. 50 g of LLDPE pellets incorporated 1%, 5%, and 10% (w/w) of lysozyme and mixed lysosome and glucose oxidase were examined, respectively. Pure LLDPE was used as a control.

Sample characterizations

The study of the structural and morphological properties

The morphological image of the polyethylene films was determined using a Digital microscope Bresser LCD 50x–2000x "Bresser GmbH" (LCD Microscope V5.0, Gutenbergstr, 2-46414 Rhede, Germany). Nozzle type is digital display. The objective of the microscopy lenses was 4x, 10x, and 40x revolving device.

Vapor permeability analysis

The determination of the vapor permeability of polymer materials was carried out using the "PERME W3/030" device by GOST GB1037. The studies were carried out at a temperature of 38°C and a humidity of 90%. The quantification of wettability of the surface films is called contact angle and the values were recorded through the surface tension using ASTM D7334-08 standard.

Analysis of mechanical properties

The stretching of the samples was tasted through an auto tensile tester machine (model:C610H, serial number B1610V1R1-000-024008-E0343C, Labthink instruments Co., Ltd, China). Test method was based on the GOST 14236 techniques and with speed of 100 mm/min. The mechanical properties of the biocomposite film (such as tensile strength and elongation-at-break of the samples were determined by calculating the average and standard deviation of the slices. Twelve longitudinal and transverse extrusion direction replicated slices were recorded from the center of the films.

FTIR spectroscopy analysis

The functional group of the produced composite is used to determine the structural configuration. The FTIR spectrometer (FTIR-990IN, Instrument SN: ZKRMFIK0 O_2, Tianjian Labor Scientific Instrument Co., Ltd., China) was used to determine the functional groups. The working environment humidity is 15-60% and the system has been tested with a resolution of 4 cm in 1, a temperature of 30.5°C, 2 apodization triangles and a collection time of 20 min. The

absorption spectra of the FTIR were collaborated between 4000 to 400 cm^{-1} wavenumber.

RESULTS AND DISCUSSION

Appearance of the resulting film samples by flat-slot extrusion

Four LLDPE films with good technical specifications and no flaws were successfully obtained (Figure 1): pure LLDPE, with 1%, and 5% w/w lysozyme, as well as with 1% w/w lysozyme and glucose oxidase. An LLDPE film with 5% lysozyme and glucose oxidase was obtained with defects (holes, uneven surface). At a concentration of 10% for lysozyme and 10% for the mixture of lysozyme and glucose oxidase, we were unable to obtain films. Small, intermittent, thick, solid, dark-colored pieces are obtained. Lysozyme and glucose oxidase affected the color of the resulting films and reduced the transparency of the polyethylene films clearly, noting that glucose oxidase colored the film yellow more than lysozyme. In addition, it was found that when additives were introduced into the polymer matrix at more than 5%, a smooth and inelastic

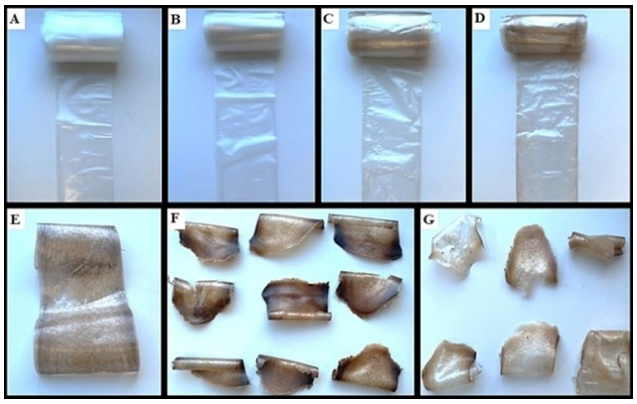

Figure 1. The external appearance of the obtained films: A) LLDPE film, B) LLDPE film with lysozyme 1%, C) LLDPE film with lysozyme 5%, D) LLDPE film with lysozyme and glucose oxidase 1%, E) LLDPE film with lysozyme and glucose oxidase 5%, F) LLDPE film with lysozyme 10%, G) LLDPE film with lysozyme and glucose oxidase 10%.

polymer film was obtained. Therefore, further studies were conducted on polymer films with the ratios given below: LLDPE film, LLDPE film with lysozyme 1%, LLDPE film with lysozyme 5%, LLDPE film with lysozyme and glucose oxidase 1%, LLDPE film with lysozyme and glucose oxidase 5%.

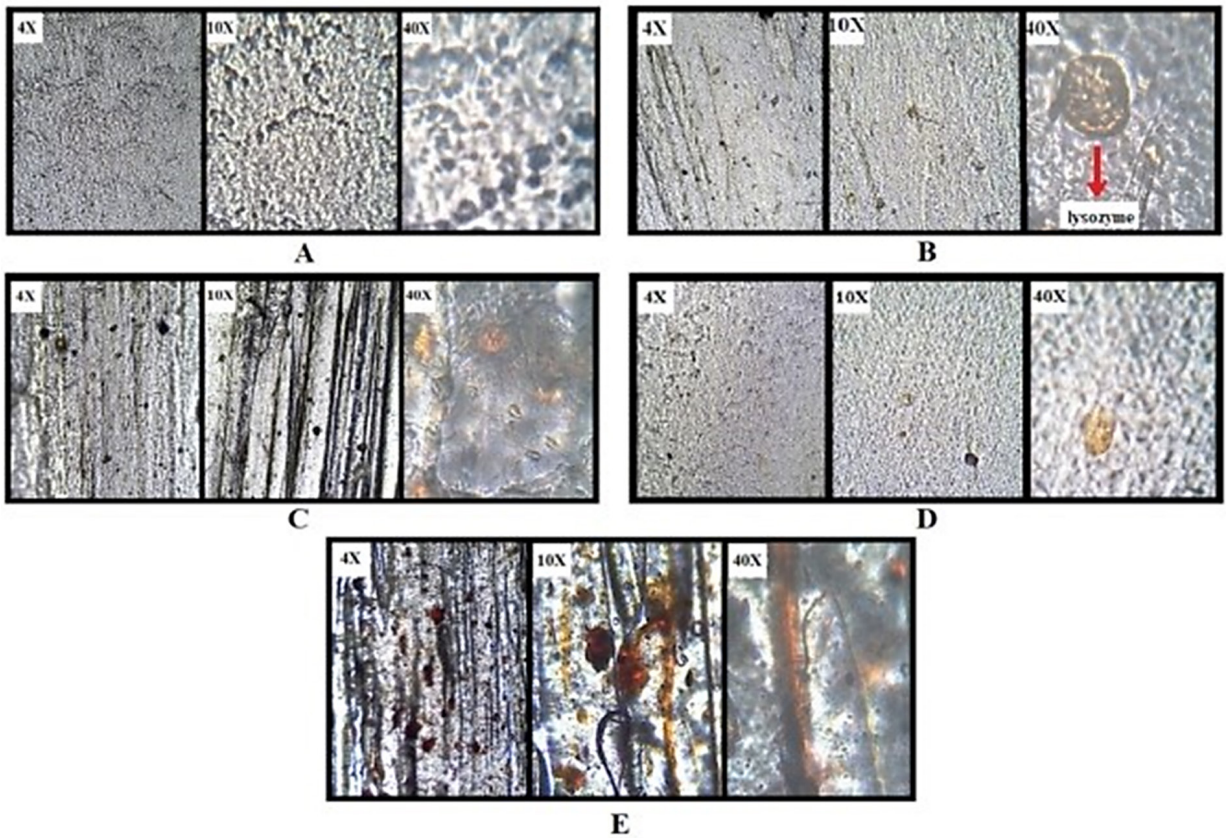

Figure 2. The structure of polymer materials under the microscope at different magnifications: 4x, 10x, 40x. A) LLDPE film, B) LLDPE film with lysozyme 1%, C) LLDPE film with lysozyme 5%, D) LLDPE film with lysozyme and glucose oxidase 1%, E) LLDPE film with lysozyme and glucose oxidase 5%.

Morphological analysis of the sample

The morphological image of the LDPE/lysozyme/glucose oxidase composite film is displayed in Figure 2. Magnifications of 4x, 10x, and 40x were used. At low content of enzymes added to the polyethylene polymer matrix, the biocomposite film showed good compatibility and homogeneity. This is mainly due to the uniform dispersion of the enzymes on the surface of the polymer matrix. The films' compatibility decreased with increasing enzyme concentrations (5 and 10%w/w), nonetheless, as a result of the uneven distribution of enzymes within the LLPE polymer matrix. The distribution of additives in the structure of the LLDPE material is shown in Figures 2(B, C) at 40x magnification. In Figure 2(D, E) at 10x magnification, it is possible to notice two circular objects of different colors. The light brown color is most likely due to lysozyme and the dark brown color is due to glucose oxidase, because before exposure to the high heat necessary for the extrusion process and obtaining the films, the color of lysozyme was white and the color of glucose oxidase was light brown.

Vapor permeability of the samples

The WVTR and contact angle of bio-composite films are presented in Figure 3 and Table 1. In general, the value of WVTR varies with the concentration of enzymes. At a concentration of 1% for lysozyme and 1% for the mixture of lysosome and glucose oxidase, the permeability decreased by 1.8565 g/m^2.24h compared to the control sample. With 5% concentration of lysozyme, the water vapor permeability was identical to the control sample. At a 5% concentration of the mixture of lysozyme and glucose oxidase, the vapor permeability increased approximately 2.6 times compared to the control sample. Besides, the additive of glucose oxidase enzymes has shown significant effect on the biocomposite water vapor permeability. Hence, biocomposites with high concentration of enzymes (lysosome and glucose oxidase) have weak barrier properties.

The hydrophilic groups in the bio-composite material are responsible for the films' hydrophobic and hydrophilic characteristics. According to Table 1, the contact angle of enzyme with 1%w/w and mixture

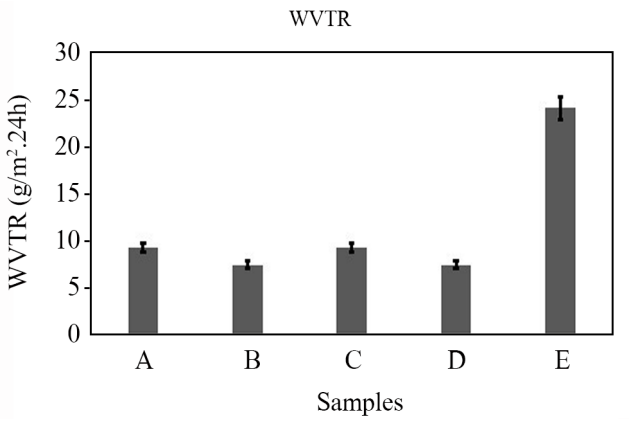

Figure 3. Results on the vapor permeability of the enzyme based LLDPE films. Note: A) LLDPE film, B) LLDPE film with lysozyme 1%, C) LLDPE film with lysozyme 5%, D) LLDPE film with lysozyme and glucose oxidase 1%, E) LLDPE film with lysozyme and glucose oxidase 5%.

of lysozyme and glucose oxidase with 5%w/w was observed to be 55° and 47°, respectively. It was evident that the inclusion of enzymes somewhat reduced the contact angle of polyethylene-based films. This is mostly because the enzyme has a lot of hydrophilic groups on its surface. Four sets of enzymes in the LLDPE polymer matrix with varying enzymatic contents all clearly exhibited the water vapor barrier effect as compared to the pure LLPDE. However, the film's water vapor barrier performance declined as the concentration of enzymes grew because the film's water vapor transmission rate increased as well. Enzymes have a high capacity for water absorption due to their abundance of hydrophilic groups. Larger gaps between molecular chains are created as water molecules flow through the composite film, increasing the free volume of the molecular chains and facilitating the passage of water vapor. The water vapor barrier performance of the LLDPE-based composite films can therefore be improved by adding a specified quantity of enzymes.

Physical and mechanical characteristics

The physical and mechanical properties of the obtained samples are presented in Figures 4 and 5. According to Figure 4, in the longitudinal direction, lysozyme at a concentration of 1% showed no significant effect on the tensile strength (18.2 MPa) compared to the

Table 1. Contact angle of the bio-composite films.

	Control	Lysozyme (1%)	Lysozyme + Glucose oxidase 1%	Lysozyme (5%)	Lysozyme + Glucose oxidase (5%)
Angle (°)	70	55	52	50	47

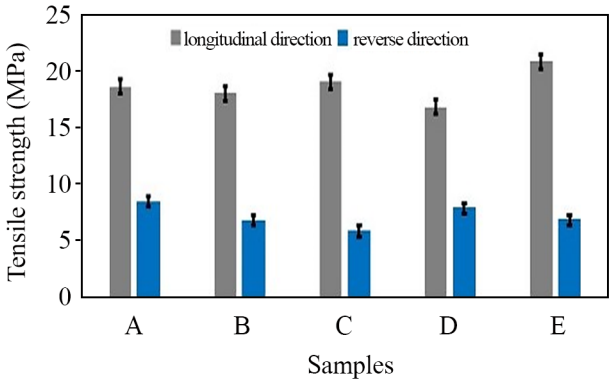

Figure 4. Tensile strength of LLDPE with enzymes (longitudinal and transverse directions). Note: A) LLDPE film, B) LLDPE film with lysozyme 1%, C) LLDPE film with lysozyme 5%, D) LLDPE film with lysozyme and glucose oxidase 1%, E) LLDPE film with lysozyme and glucose oxidase 5%.

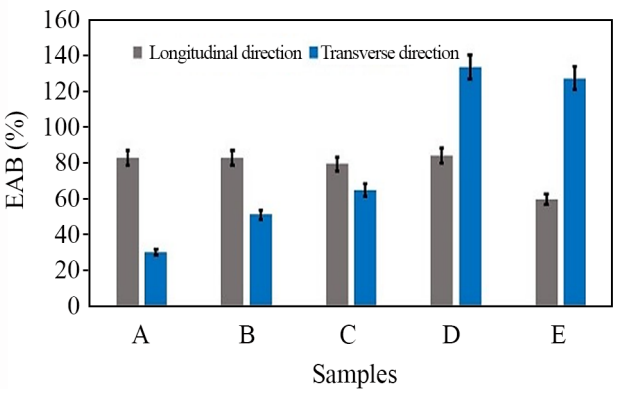

Figure 5. Elongation-at-break of LLDPE with enzymes (longitudinal and transverse directions). Note: A) LLDPE film, B) LLDPE film with lysozyme 1%, C) LLDPE film with lysozyme 5%, D) LLDPE film with lysozyme and glucose oxidase 1%, E) LLDPE film with lysozyme and glucose oxidase 5%.

control sample (18.8 MPa). The same thing happens at the same concentration for the mixture of lysozyme and glucose oxidase (17 MPa). At a 5% concentration of lysozyme, mixture of lysozyme and glucose oxidase, the tensile strength showed an increment and the values were recorded as 19.22 MPa and 21 MPa, respectively. A greater effect of glucose oxidase on tensile strength is observed compare to lysozyme. In the transverse direction, the addition of lysozyme and glucose oxidase at all concentrations slightly reduced the tensile strength. The strength decreased with increasing concentrations of additives. Lysozyme showed a greater ability to reduce tensile strength compared to glucose oxidase.

The elongation-at-break of the biocomposite films is presented in Figure 5. Overall, the elongation of the films was almost the same with pure LDPE, while it decreased with increasing lysosome and glucose oxidase concentrations. Besides, in the longitudinal direction, the addition of lysozyme and a mixture of lysozyme with glucose oxidase at a concentration of 1% and lysozyme at a concentration of 5% showed a negligible effect on elongation-at-break. While the addition of a mixture of lysozyme and glucose oxidase at concentrations of 5% significantly reduced the elongation of polyethylene polymer matrices. However, in the transverse direction, the elongation of the layers showed a significant increase with increasing enzyme concentration. In contrast, the elongation of the LDPE films containing a combination of the enzymes lysozyme and glucose oxidase was greater than that of lysozyme alone at concentrations of 1% and 5%. In conclusion, altering the enzymes to LLDPE ratio is necessary to increase the elongation upon film break. Hence, the ratio of plasticizers or fillers to the polymer matrix is one of the key factors for increasing the elongation-at-break of the films [28].

FTIR spectroscopy

The results of FTIR spectroscopy are presented in Figure 6 [A) LLDPE of Library (FTIR), B) LLDPE obtained in the laboratory (control), C) LLDPE film with lysozyme 1%, D) LLDPE film with lysozyme 5%, E) LLDPE film with lysozyme and glucose oxidase 1%, and F) LLDPE film with lysozyme and glucose oxidase 5%]. The peaks are formed approximately at 570, 1500, 2700, and 3000 cm^{-1}. The non-smooth

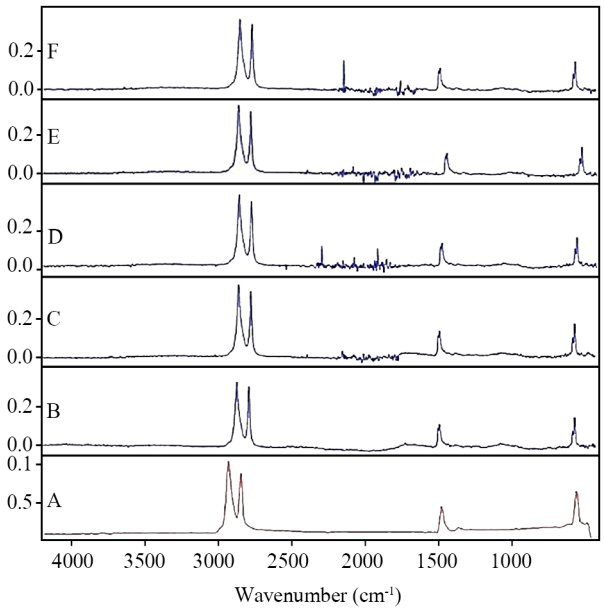

Figure 6. Results of FTIR spectroscopy for LLDPE with enzymes.

line is displayed between 1700-2400 cm^{-1} starching due to the triple bonds of the functional groups in the film. This indicates the triple bond of nitrites (C-N), O-H, and carbines(C-C) groups. Additionally, peaks are formed at 2200 and 3000 cm^{-1} in composite samples D and F [29]. Next to this, the non-smooth line is displayed in all sample composites except B compared to the control.

Additionally, C, D, E, and F showed asymmetrically peak forms, and in B no peak form in the carbon-to-carbon triple bond. Therefore, it confirmed that there is an effect of the lysozyme and glucose oxidase on the LLDPE composites. Having studied polymer materials using IR spectroscopy, it can be noted that the IR spectra (in Fig. 6) in the wavenumber range from 1700 to 2400 cm^{-1} show the additives introduced into the samples under study.

CONCLUSIONS

Bio-composites (linear low-density polyethylene, lysozyme, and glucose oxidase) were synthesized in this study. In this study, 50 g of LLDPE pellets were fixed with 1%, 5%, and 10% (w/w) lysozyme and glucose oxidase. Research on LLDPE composite enzyme is not yet developed. The mechanical properties such as tensile strength and elongation-at-break, barrier properties such as water vapor permeability, and morphological structure LLDPE/lysozyme/glucose oxidase were examined. The morphological surface of the composites was highly concerned by the enzyme concentrations. In this line, all samples were smooth and no void areas were observed. However, As the enzyme concentration increased, the biocomposite layers became more brittle and stiffer than the others. Besides, high concentration (10%) of lysozyme and mixtures of lysozyme and glucose oxidase negatively affected the technological properties of the films obtained using the extrusion technique. Though the permeability rose by around 2.5 times when a mixture of lysozyme and glucose oxidase was added at a concentration of 5%, films with good characteristics could still be obtained up to a 5% concentration of lysozyme and lysozyme and glucose oxidase. In general, LLDPE with a lower enzyme ratio has mechanical and barrier qualities that are practically better than those of pure LLDPE.

CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest.

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