Utilization of Various Lignocellulosic Substrates for *Pleurotus ostreatus* Mushroom Cultivation in the Manufacturing of Polycaprolactone (PCL)-based Biocomposite Films

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The possibility of using beech wood sawdust and hazelnut husk waste generated during the cultivation of *Pleurotus ostreatus* mushrooms as a filler in polycaprolactone (PCL)-based biocomposite films was investigated. Chemical and physical properties of the PCL biocomposites were determined. The beech sawdust and hazelnut husk were exposed to degradation for 73 d and 78 d, respectively, in slightly acidic environments at a relative humidity of 75%. The degraded materials caused the holocellulose and lignin contents in the PCL biocomposites to decrease, while the cellulose and α-cellulose contents increased. In general, as the lignocellulosic waste content increased, the tensile strength (TS) and elongation at break (EatB) values decreased and the tensile modulus (TM) and water absorption (WA) values increased. It was determined that the PCL biocomposite with the degraded beech sawdust absorbed more water than the composite with the undegraded beech sawdust. On the other hand, the PCL biocomposite with the degraded hazelnut husk absorbed less water than the composite with the raw hazelnut husk.

Keywords: Mushroom; Polycaprolactone (PCL); Film; Pleurotus ostreatus

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INTRODUCTION

It has become increasingly important to use natural and readily available resources to develop suitable and beneficial products. The production of cultivated mushrooms has gained attention because of the nutritional value and sustainable attributes mushrooms possess. The *Pleurotus ostreatus* mushroom is the second most cultivated edible mushroom in the world. *P. ostreatus* has medical benefits that make it an economically important material (Sánchez 2010; Waktola and Temesgen 2020). The *P. ostreatus* mushroom can deteriorate lignocellulosic materials. This behavior in mushrooms is known as white rot (Bari *et al.* 2015). The mushroom micelles wrap the lignocellulosic substrates and secrete enzymes that break down the polymers in the substrate. This causes the organic components to degrade over time as the substrate develops fungal biomass in the polymers (Appels *et al.* 2019). After mushrooms are harvested, a large amount of degraded waste compost fiber is generated that has no specific use in industry.

The recycling of waste material has important environmental, engineering, economical, and social implications for ensuring sustainable resource management in the world (Ng *et al.* 2014). High polymer utilization causes serious environmental problems due to the large amount of polymeric waste that is thrown away (Moreno and Saron 2017). Recycled polymers can be used in many applications, such as furniture, packaging,

automation, marine, deck floors, fences, door and window frames, *etc*. However, after these items are replaced, they are often discarded rather than recycled, which is contributing to environmental concerns (Hu and Lim 2007; Karakus *et al.* 2016). To reduce the amount of waste that enters the environment, biodegradable polymers have gained preference over non-biodegradable polymers for some applications such as packaging. Polycaprolactone (PCL) is one of the biodegradable plastics that is used in various applications with lignocellulosic compounds (Dong and Davies 2011; Ermeydan *et al.* 2014; Dhakal *et al.* 2018).

This study evaluated the potential utilization of hazelnut husk and beech wood as filler materials in the manufacturing of PCL-based biocomposite films. For comparison, undegraded fiber composite films with no mushroom cultivation were also produced. A chemical analysis of the fibers was also conducted. The physical properties such as the density and water absorption (WA), and the mechanical properties such as the tensile strength (TS), the tensile modulus (TM), and the elongation at break (EatB) values of the PCL biocomposite films were also determined.

EXPERİMENTAL

Materials

The beech sawdust and the hazelnut husk materials were obtained from timber factories and hazelnut producers in Düzce, Turkey. These lignocellulosic materials were granulated into powdered form using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Before biocomposite manufacturing, the powdered beech sawdust and the hazelnut husk samples were screened though 40, 60, 80, 100, and 200 mesh-size screens. The screened powders were then were dried in the oven at 103 °C for 24 h before they were made into biocomposites. The PCL (CAPA 6500; Perstorp Ltd., Warrington, UK) with a number average molecular weight of 50,000 g/mol was used as the polymer matrix.

Methods

Degradation process

The ground beech sawdust and hazelnut husk samples were used for the mushroom treatment. The *P. ostreatus* mushroom culture was grown on malt agar and stored in an incubator at 25 °C for 3 w. Before the inoculation, the beech sawdust and hazelnut husk samples were irrigated with tap water for 3 w to increase the humidity level, as determined by the palm test methods (Kwon and Kim 2004). The samples were placed in 1 kg heat-resistant bags and then sterilized in an autoclave at 90 °C for 90 min. Mushroom production was tested in 1 kg bags in 5 repetitions. After they were sterilized, the samples were cooled and inoculated. For the spawn run, the incubated beech and hazelnut husk samples were put in a room at a temperature of 25 °C and a relative humidity of 90%. After the mycelium was completed, the substrates were removed from the bags and placed in a room at 18 ± 2 °C to obtain the final product. A single harvest was made from the mushrooms that were developed on the beech sawdust and hazelnut husk. The mushroom waste on the samples was cleaned, washed, and dried. The time that elapsed in each phase was recorded.

Chemical analysis for lignocellulosic wastes

The chemical analysis was performed on the beech wood and hazelnut husk flours before and after the degradation process. The holocellulose and cellulose contents were

determined by the chlorite method (Wise and Karl 1962) and the Kurschner and Hoffer (1969) method, respectively. The α-cellulose content and the lignin content were calculated by the TAPPI methods T203 (1988) and T222 (1971), respectively. Chemical ingredients were replicated 10 times.

Biocomposite manufacturing

During the manufacturing process, the PCL and the lignocellulosic materials (neat and degraded beech wood and hazelnut husk), were dried in a conventional oven to reduce their moisture content to less than 1%. The panels were manufactured based on the experimental design in Table 1 and Fig. 1. The ingredients were first mixed in a high-intensity mixer for homogenous blending.

Table 1. Experimental Design

ID	Filler	Polymer Content
PCL (P)	-	100%
P-5-B	5% beech wood (B)	95%
P-5-DB	5% degraded beech wood (DB)	95%
P-10-B	10% B	90%
P-10-DB	10% DB	90%
P-5-H	5% hazelnut husk (H)	95%
P-5-DH	5% degraded hazelnut husk (DH)	95%
P-10-H	10% H	90%
P-10-DH	10% DH	90%

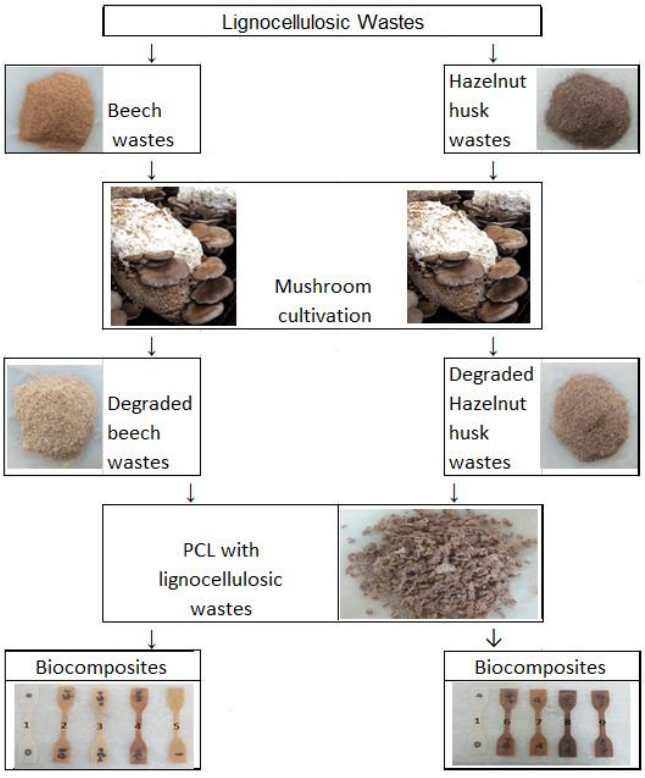


Fig. 1. Biocomposite material manufacturing scheme

The mixtures were compounded in a laboratory scale single screw extruder (IBB 30; Teknomatik, Istanbul, Turkey) at a screw speed of 40 rpm and a temperature range of 70 °C to 110 °C. The extrudates were collected, cooled, and granulated into pellets with an HDX injection molding machine (HAIDAHDX-88, Ningbo Haida Plastic Machinery Co., Ltd., Ningbo, China). The pellets were dried in an oven before the films were manufactured. Finally, certain quantities of pellets were placed into molds, and the PCL films were manufactured by a compression molding press with cooling capabilities (Sistem Machinery, Kahramanmaras, Turkey). The maximum pressure, maximum temperature, and maximum compression time was 150 bar, 120 °C, and 10 min, respectively (mol dimensions: 0.5 mm thick × 160 mm wide × 160 mm long). The mechanical properties of the manufactured films were determined according to the ASTM standard D638 (2010). The tensile strength (TS), tensile modulus (TM), and elongation at break (EatB) values were determined with a ZwickRoell 10 KN test machine (Ulm, Germany). The results were evaluated by the analysis of variance (ANOVA) test using Design-Expert, version 7.0.3 software (Stat-Ease, Minneapolis, MN, USA). Mechanical testing of biocomposites was replicated 10 times.

RESULTS AND DISCUSSION

The media features of the wastes and their deterioration times are given in Table 2. Both the beech wood and hazelnut husk had similar moisture contents and pH values. The spawn run time of the beech wood was shorter than that of the hazelnut husk. Both media had the same harvest time but the total time was higher for the hazelnut husk due to its longer spawn run time. The high extractives content in substrates has been found to limit the growth of mushroom micelles (Mahesh and Satish 2008; Mutai *et al*. 2009; Cruz-Lopez *et al*. 2012). The longer spawn time of the hazelnut husk may have been due to its higher tannin content. In a study, it was reported that the tannin limits fungal growth (Lopes *et al.* 2020). The moisture content of the substrates is also a very important factor in fungal mycelium development (Wang *et al*. 2001). In previous studies, it was emphasized that the moisture content of the environment should be between 43% and 75% (Wang *et al.* 2001; Hernández *et al*. 2003). The *P. ostreatus* in this study were cultivated in a pH range between 6.38 and 6.65, similar to the pH range used by Yildiz *et al*. (2002).

Table 2. Media Features and Deterioration Times of the Wastes

Waste	Ratio	Relative Humidity (%)	pH	Spawn Run Time (d)	Harvest Time (d)	Total Time (d)
Beech	100%	75 ± 5%	6.38 ± 1	26	47	73
Hazelnut husk	100%	75 ± 5%	6.65 ± 1	31	47	78

Table 3 shows the chemical composition (holocellulose, cellulose, α-cellulose, lignin) of the beech sawdust, the degraded beech sawdust, the hazelnut husk, and the degraded hazelnut husk materials. The beech sawdust was found to have the highest holocellulose content (74%). The holocellulose content in the degraded beech waste decreased to 71% due to the fungal degradation. The holocellulose contents of the hazelnut husk and the degraded hazelnut husk were 57% and 51%, respectively. Previous studies

have reported holocellulose contents of 73% and 55% in beech sawdust and hazelnut husk, respectively (Güler 2015; Ateş *et al.* 2016). The beech sawdust had a higher cellulose content than the hazelnut husk, and the cellulose ratio in the holocellulose increased due to the degradation caused by the fungal enzymes. The beech sawdust had the highest α-cellulose to cellulose ratio. The α-cellulose content in the beech sawdust and the hazelnut husk increased with degradation by the fungal enzymes. The highest lignin content was determined in the hazelnut husk (35%) compared to the beech wood (24%). The lignin content in the degraded beech sawdust and hazelnut husk was reduced further. Ateş *et al.* (2016) also reported decreased lignin and increased cellulose contents in beech wood exposed to *Pleurotus* degradation. Cruz-Lopez *et al*. (2012) reported higher tannin contents in hazelnut husk compared to beech wood.

Table 3. Chemical Composition of the Lignocellulosic Wastes

Compound	Beech Sawdust (%)	Degraded Beech Sawdust (%)	Hazelnut Husk (%)	Degraded Hazelnut Husk (%)
Holocellulose	74.22 (0.4)*	71.27 (0.17)	57.11 (0.96)	51.40 (0.20)
Cellulose	47.07 (1.35)	49.97 (0.79)	29.52 (1.26)	31.02 (0.59)
α-Cellulose	42.86 (0.07)	45.69 (0.50)	23.19 (7.48)	26.97 (7.21)
Lignin	23.55 (1.39)	21.49 (0.26)	35.21 (0.25)	32.39 (0.27)

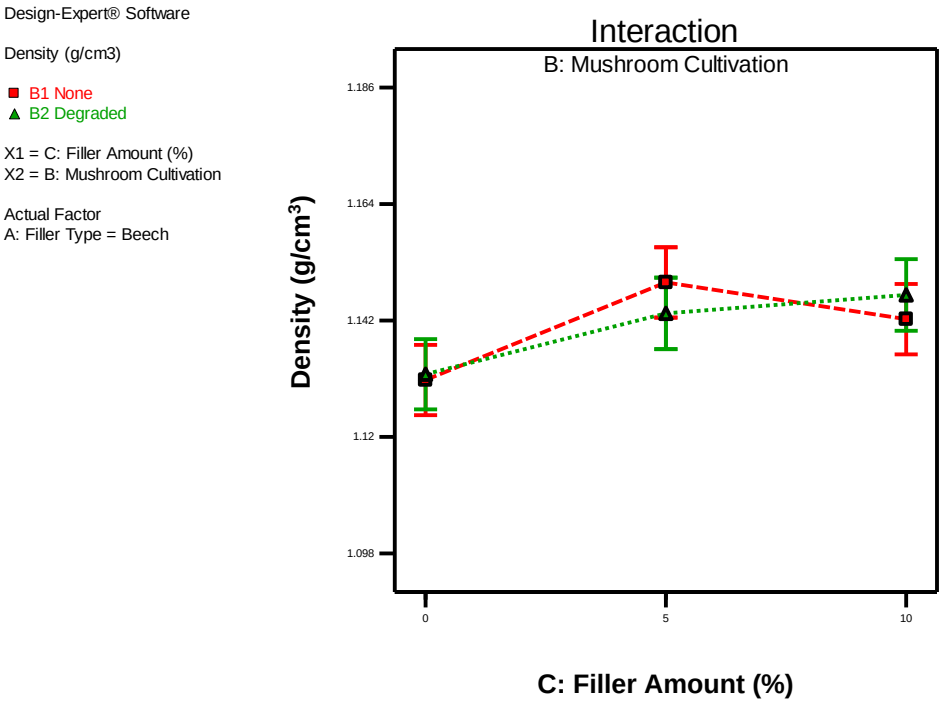
*The values in parentheses indicate the standard deviation

Table 4. Density and the Mechanical Properties of the Biocomposites

ID	Density (g/cm^3)	TS (MPa)	TM (MPa)	EatB (%)
P	1.13 (0.02)*	15.62 (1.13)	485.5 (68.9)	335 (64.0)
P-5-B	1.15 (0.02)	13.53 (0.97)	521.0 (36.9)	23.9 (14.4)
P-5-DB	1.15 (0.04)	14.15 (0.85)	513.8 (94.4)	18.8 (12.9)
P-10-B	1.14 (0.01)	12.15 (1.11)	551.6 (29.2)	12.1 (9.2)
P-10-DB	1.14 (0.02)	11.77 (0.86)	601.0 (63.4)	16.2 (7.0)
P-5-H	1.14 (0.02)	11.60 (1.37)	509.2 (46.2)	19.8 (14.6)
P-5-DH	1.14 (0.03)	13.49 (0.64)	516.4 (94.4)	34.9 (21.0)
P-10-H	1.15 (0.05)	10.29 (1.29)	565.9 (44.1)	18.8 (8.0)
P-10-DH	1.16 (0.05)	11.86 (0.66)	614.3 (60.2)	19.2 (4.2)

*The values in parentheses indicate the standard deviation

In this study, the effect of the degradation, the filler type (beech wood *vs.* hazelnut husk), and filler contents (0%, 5%, and 10%) on the density and mechanical properties of the PCL based biocomposite films were investigated. The mean values and the standard deviations are presented in Table 4. The interaction graphs of the samples are shown in Figs. 2a and 2b. The statistical analysis showed that the filler type and the degradation did not have a significant effect on the density values of the composites. The p-values for the beech wood and hazelnut husk materials were 0.123 and 0.688, respectively. However, the filler content did have a significant effect on densities of the composites ($P = 0.005$) between the 0% and 10% addition levels.



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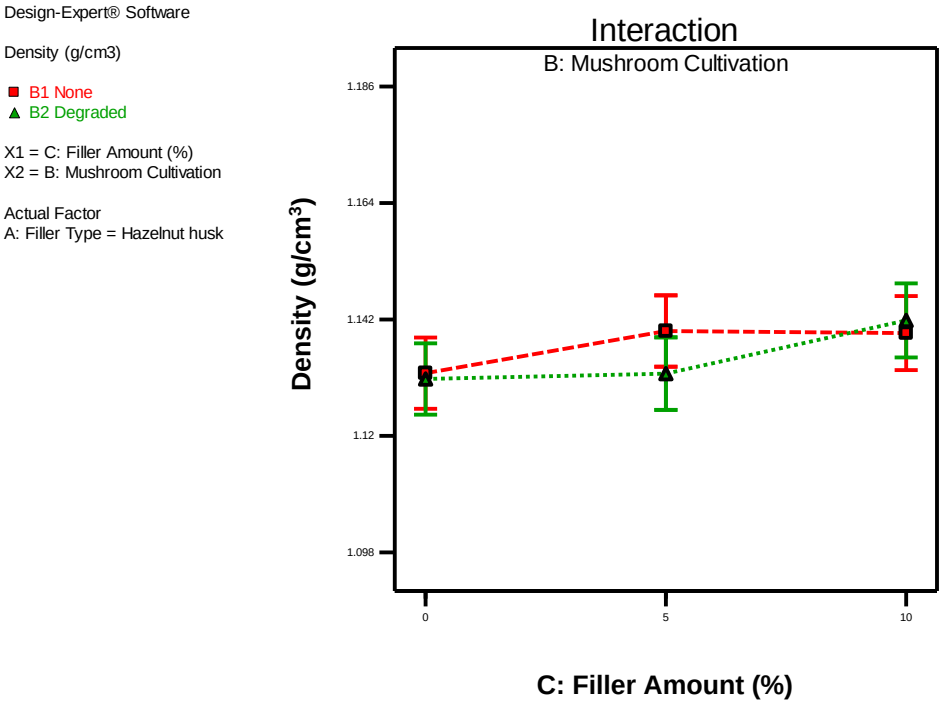

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Fig. 2. The interaction graphs of the density (g/cm^3) properties for the PCL composites (a): beech wood, (b): hazelnut husk

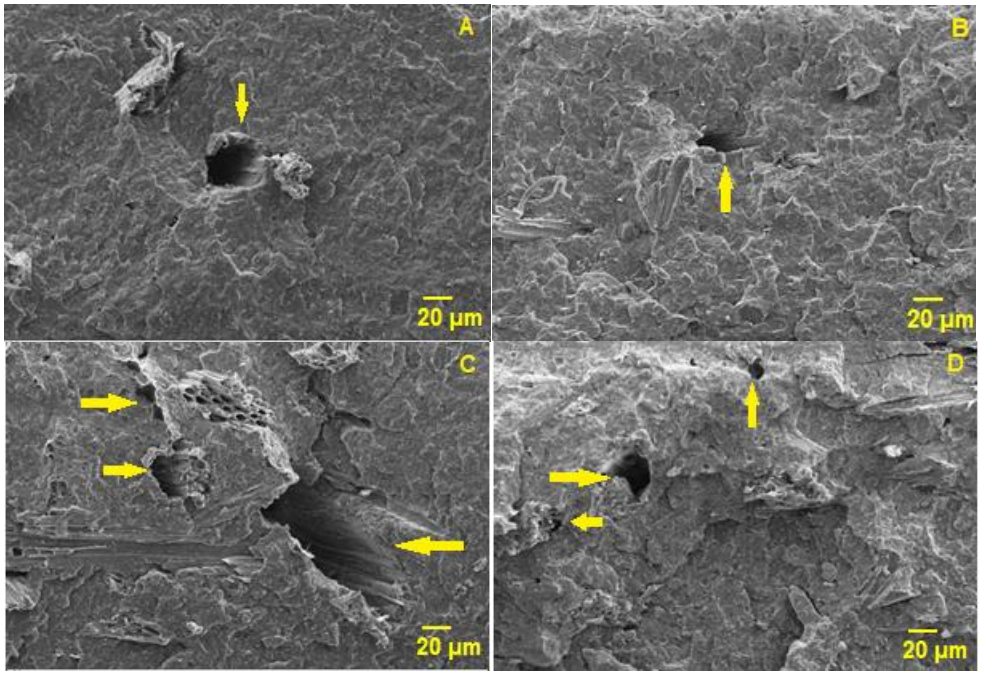


Fig. 3. SEM images of fillers and polymer composites: A) P-5-B; B) P-5-DB; C) P-10-B; and D) P-10-DB

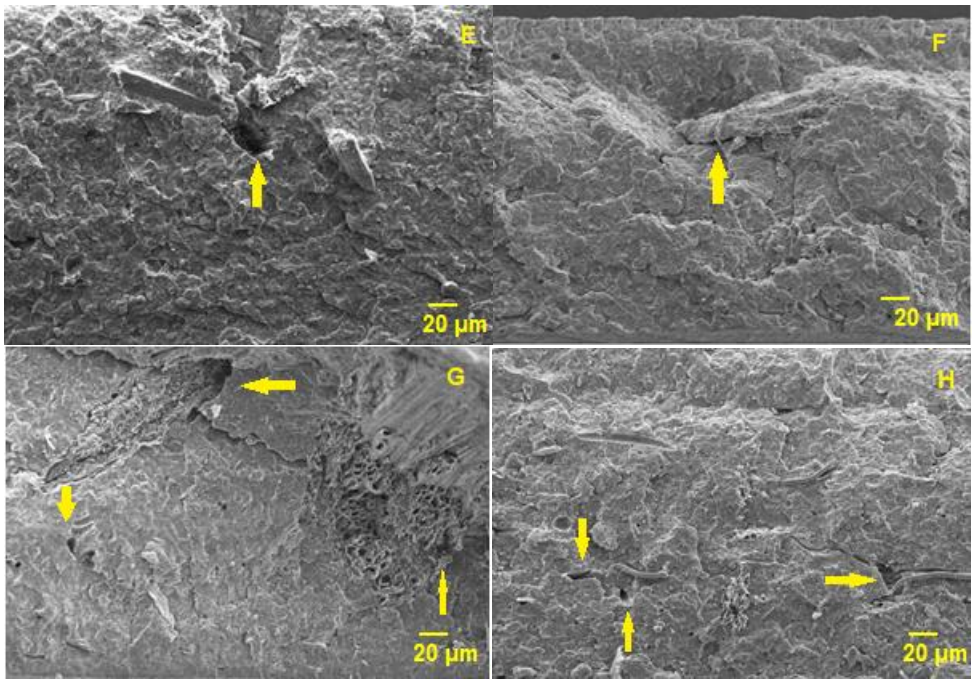


Fig. 4. SEM images of fillers and polymer composites: E) P-5-H; F) P-5-DH; G) P-10-H; and H) P-10-DH

As can be seen in Table 4, the statistical analysis showed that the filler type, the degradation level, and the filler content all had significant effects on the TS values of the biocomposite films ($P = 0.0003$, $P = 0.0026$, and $P < 0.0001$, respectively). The interaction graphs are presented in Figs. 5a and 5b. The filler content had a large influence on the TS, as the addition of lignocellulosic filler reduced the TS due to the lack of compatibility between the non-polar polymer and the polar lignocellulosic filler (Figs. 3 and 4). A coupling agent was not used in this study, so these results were expected. The lack of

adhesion between the hydrophobic polymer matrix and the hydrophilic filler may have caused this reduction in strength due to the limited stress transfer (Mengeloglu and Karakus 2008). A similar strength reduction with the use of wheat straw flour was also reported for PCL based composites by Mengeloglu and Karakus (2012). Reduced TS values for composites with various polymer and lignocellulosic filler mixtures were reported in other studies (Colom *et al*. 2003; Huda *et al*. 2006). The biocomposites that were produced with the beech wood had statistically similar TS values, regardless of the degradation process. However, higher TS values were recorded when degraded filler was utilized in the biocomposites that were produced with the hazelnut husk. It was difficult to determine how the degradation process changed the composite properties since the mushroom cultivation period was approximately 30 d. Higher property changes were reported by Ayrilmiş *et al*. (2015) when longer mushroom exposure time was applied. In this study, wood flour from virgin and decayed scots pine tree treated by brown rot fungi were manufactured as polymer composites and their properties were determined. The chemical changes that occurred during the decay process were emphasized.

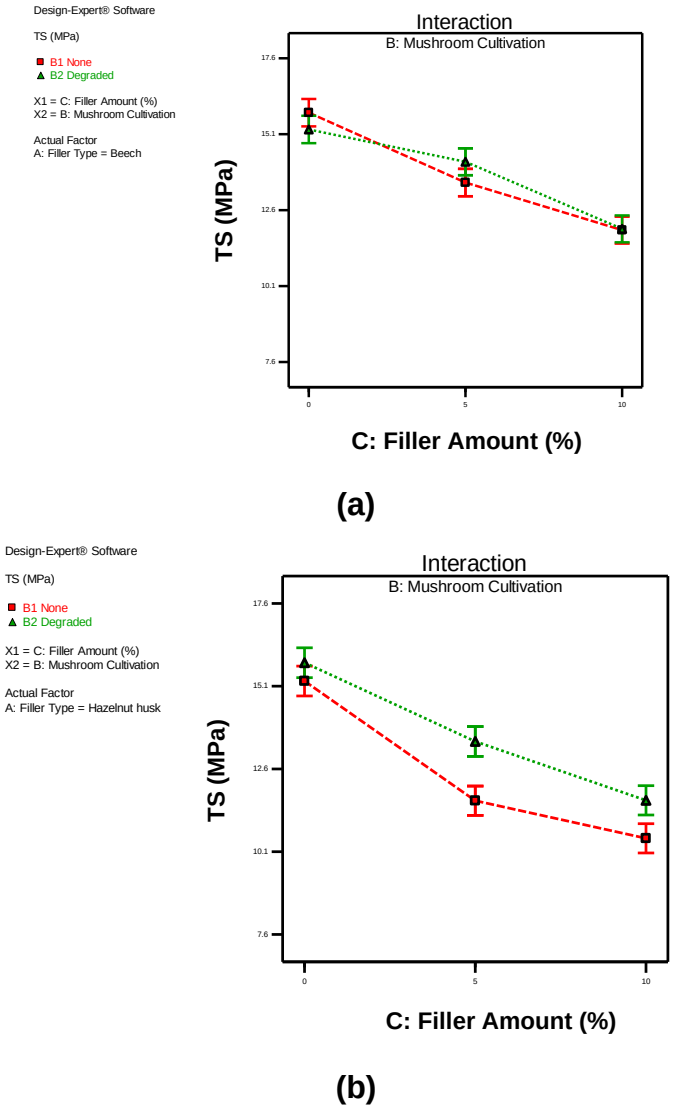
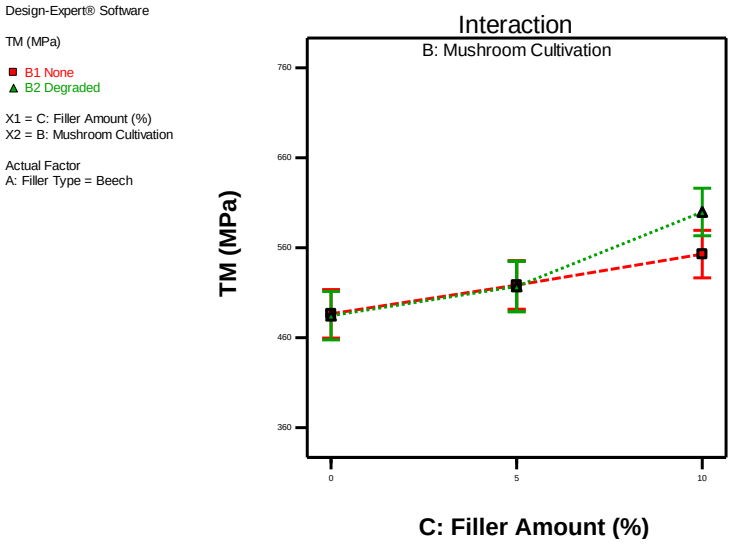


Fig. 5. The interaction graphs of the TS properties for the PCL composites (a): beech wood, (b): hazelnut husk

The filler content was the only significant factor ($P < 0.0001$) in the TM properties of the biocomposites. The TM values increased as the filler content increased, regardless of the filler type or the degradation level. The p-values for the beech wood and hazelnut husk materials were equal to 0.7800 and 0.1384, respectively (Fig. 6a and Fig. 6b). Dhakal *et al*. (2018) found that the TM properties in PCL composites also increased with the addition of 20% palm fiber. Generally, thermoplastic based lignocellulosic composites provide higher TM values compare to neat polymers. Some studies have concluded that this is due to the rule of mixture (Bhaskar *et al.* 2011; Ayrilmiş *et al*. 2015). When a lignocellulosic filler with a higher modulus value is mixed with a thermoplastic that has a lower modulus value, the resulting composite modulus is higher than that of the polymer modulus. Even the small filler content that was used in this study significantly increased the TM values.



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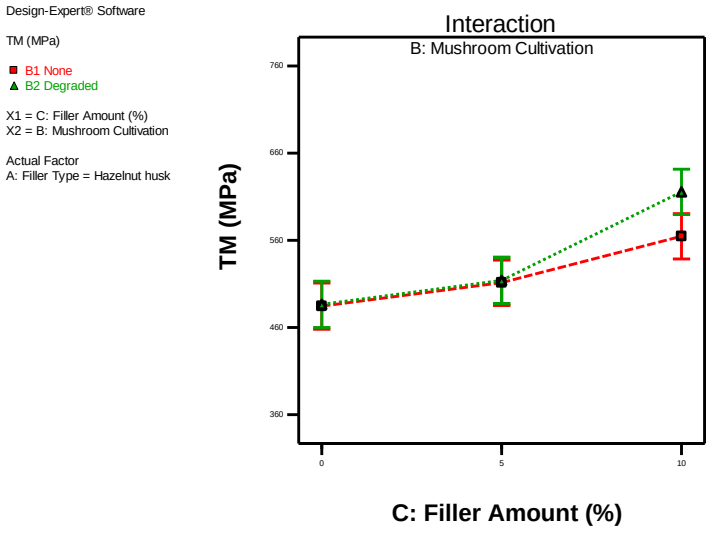

(b)

Fig. 6. The interaction graphs of the TM properties for the PCL composites (a): beech wood, (b): hazelnut husk

The EatB values of the biocomposites are presented in Table 4. According to the statistical analysis, the filler content had a significant effect on the EatB value ($P < 0.01$). The addition of 5% and 10% filler significantly reduced the EatB values. The PCL composites had lower EatB values than the biocomposite samples, regardless of the filler type or the degradation level. The EatB values were not significantly impacted by the filler type ($P = 0.6366$) or the degradation level ($P = 0.7430$). Other studies reported lower EatB values with the addition of lignocellulosic filler in PCL composites (Ludueña *et al.* 2012; Mengeloglu and Karakus 2012; Dikobe and Luyt 2017).

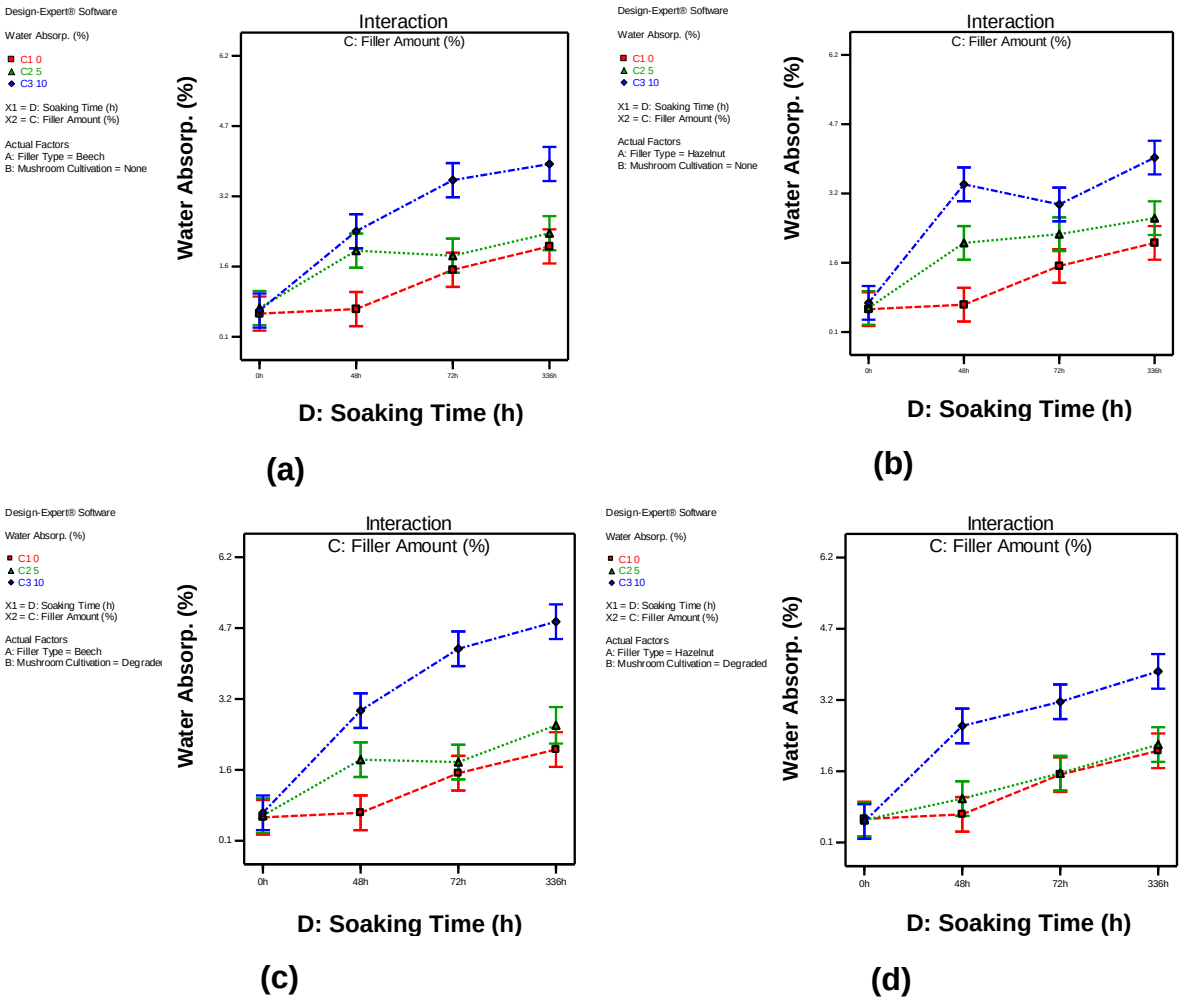

Fig. 7. The interaction graphs of the WA properties for the PCL composites (a): beech wood, (b) hazelnut husk, (c): degraded beech, (d): degraded hazelnut husk

The interaction graphs of the WA values for the PCL biocomposites are presented in Fig.7. The addition of the lignocellulosic waste filler in the PCL matrix increased the WA values significantly ($P < 0.0001$), regardless of the filler type or the degradation level. Filler types and degradation had no significant changes on WA values (filler types: $P=0.1210$ and degradation: $P=0.6179$). Regardless of whether the lignocellulosic wastes added to the composite are different or degraded, the lignocellulosic fiber added to the biofilm increased the WA value. The water absorption in biocomposites depends on multiple factors such as the fiber content, the fiber shape, the chemical composition of the

lignocellulosic fibers, and the compatibility of the polymers and the fibers (Vilaplana *et al*. 2010). There were obvious changes in the chemical compositions of the biocomposites in this study, but the studied 5% and 10% filler addition rates were not high enough to see the to fully materialized the effect on the properties.

CONCLUSIONS

1. The polycaprolactone (PCL)-based biocomposite films that were produced with the degraded beech wood and hazelnut husk flour yielded similar results to the films that were produced with the undegraded flour.
2. The degradation of the beech wood and hazelnut husk flour by *P. ostreatus* (white rot fungi) changed the chemical composition of the filler. The lignin content decreased while the cellulose content increased slightly.
3. The changes in the chemical composition of the PCL biocomposite properties did not significantly alter the composite properties. The 5% and 10% filler addition rate that was utilized might inhibit the materialization of changes in the biocomposite properties.
4. The addition of both the beech wood and hazelnut husk flours reduced the tensile strength (TS) of the biocomposites, regardless of the degradation level.
5. The tensile modulus (TM) and extension at break (EatB) values of the PCL biocomposite films increased with the addition of both the degraded and undegraded fillers.
6. Regardless of whether the lignocellulosic wastes added to the bio film were different or degraded, the lignocellulosic fiber added to the biofilm increased the WA value.

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