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PHYSICAL, MECHANICAL, MORPHOLOGICAL, BIODEGRADABILITY, THERMAL, AND CHEMICAL PROPERTIES OF RHIZOPUS OLIGOSPORUS MYCELIUM FOAM MADE WITH COCONUT AND COFFEE WASTE AS SUBSTRATES

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ABSTRACT

By knowing the dangers of using Styrofoam as food packaging, this research aims to obtain 100% biodegradable food packaging by using coconut fiber (CF) and coffee pulp (CP) as substrate formulation in fungal fermentation to produce mycelium foam with the help of *Rhizopus oligosporus* as a starter. Five ratios of CF and CP are designed for substrate formulation, categorized as an independent factor with three replications. ANOVA tests show that different level of substrate formulation has a significant influence ($P \leq 0.01$) on physical, mechanical properties, and biodegradability of biofoam. LSD test showed that mycelium foam with higher density, lower porosity, and lower water absorption is gained from substrate formulation with a higher portion of coffee pulp. Formulation K3 has maximum compressive strength and puncture test, amongst others. Thermogram illustrates that thermal degradation temperature increases with a higher percentage of coconut fiber in formulation substrations. More complex functional groups are identified with FTIR in the resulting biofoam of K3, K4, and K5, where the coconut fiber is used in higher percentages. This phenomenon shows that the fungal activities are more rapid in these three formulations.

Keywords: Biofoam, Coconut, Coffee, Eco-Friendly, Mycelium, Styrofoam, Tempeh Yeast.

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INTRODUCTION

Indonesian National Waste Data Management Information systems indicate that Indonesians produced waste up to 36,299,684.37 tons in 2023. Domestic waste reached up to 39.77% and plastic waste reached 19,17%, where there is an increase of 0.83% from 2022.[1] These increasing numbers may be due to the high usage of Styrofoam in food delivery services. Styrofoam is a type of disposable packaging that is highly sought after by ready-to-eat food producers, as it has a low price, temperature resistance, and light weight.[2] But Styrofoam is not biodegradable, since it is made of polystyrrene, an adhesive plastic consisting of benzene and styrene.[3] Burning the styrofoam waste leads to the formation of toxic gases such as styrene, carbon monoxide, polyaromatic hydrocarbons (PAHs), hydrofluorocarbons (HCFCs)[4], and microplastic contamination.[5] Styrene is unstable and frequently migrates to food due to the high temperature of food. Styrene is also undigested by humans and difficult to excrete through urine and feces, and when it accumulates in human body, will trigger cancer. Therefore, styrene and styrofoam, as their derivative products, are classified as toxic and carcinogenic compounds.[6-7] Providing biodegradable foam can reduce the usage of Styrofoam. Raw materials and production methods have a significant influence on the quality of a biofoam. Biofoam is generally made from biocomposites, since it can exploit any combination of materials, such as cellulose. Cellulose is a natural polymer that is more preferred recently due to its abundance, and is not considered food.[8] Cellulose can be used as substrate in fungal growth to produce mycelium foam for packaging materials.[9-10] Recent research reported that fungal fermentation using

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cellulose from sawdust, rice husk, rice bran, palm bunches, coconut fibers, and sugar cane bagasse.[11-12] Mycelium foam is widely used now in the construction industry for insulation[13], and it may also be used in food packaging materials.[14] The quality and structure of mycelium foam depend on the substrates and the fungal metabolism activities.[15] In some initial research on mycelium foam using 100% coconut fiber, soybean flour and tempeh yeast was fermented for 3-5 days produced the foam with water absorption of 73%, a porosity of 72.3%, a density of 0.1g/cm^3, a puncture strength value of 3.27 kg/cm^2, a compressive strength of 3.17 kPa.[16] This research aims to improve the quality of mycelium foam by addressing the gap from previous findings. Coffee pulp contains pectin and other natural fibers, which are combined with coconut fiber for substrates of *Rhizopus* oligosporus to grow and form a massive and dense mycelium within a certain length of fermentation time.[17]A complex compound of pulp coffee and coconut fiber is expected to accelerate the mycelium growth.[18] To facilitate the growth of *Rhizopus oligosporus,* 50% of high protein flour, with composed of 80% soybean flour and 20% tempeh flour is added in this research to fulfill the nutritional requirement.

EXPERIMENTAL

Materials and Methods

Fresh coconut fiber along with the husk were collected from a coconut plantation area near Padang Pariaman, Sumatra Barat, Indonesia. Fresh coffee pulp was gathered from Koperasi Solok Sirukam coffee plantation area, in Kabupaten Solok, Sumatera Barat, Indonesia. Other ingredients were bought online, such as soybean flour and tempeh flour (80 mesh, CV Sidatani Sembada, Yogyakarta, Indonesia), tempeh yeast powder (PT Aneka Fermentasi Industry, Bandung, Indonesia, expired 4 months from production date). Perforated plastic drinking cups, size 220 ml, were used for casting, and plastic wraps were used to wrap the samples during fermentation.

Experimental Design

This research will be conducted using a Non-Factorial Completely Randomized Design. The independent factor is the substrate formulation (K), which is a mixture of coconut fiber (CF) and coffee pulp (CP), is divided into 5 treatment, K1=50% CF: 50% CP, K2 = 60% CF: 40% CP, K3 = 70% CF: 30% CP, K4 = 80% CF: 20% CP, K5 = 90% CF%: 10 CP. Each level is replicated three times, then the total experiments is 15 units. The obtained data will be analyzed using SPSS 20.0 for analysis of variance (ANOVA), and a Least Significant Difference (LSD) further test will be carried out if there is an influence of an independent factor.

Preparation of Substrates

Fresh coconut fiber (CF) along with coconut husk (CH), and coffee pulp (CP) were brushed and separated from dirt and foreign materials, cleaned with water flows, strained, and sun-dried for four days, were the moisture contents varying from 8-10%. Dried CF and CH were weighed in a ratio of 1:1, ground up to 20 mesh. For CP, they were ground up to 40 mesh. CF, CH, and CP were stored at room temperature inside vacuum containers.

Fabrication of Mycelium Foam Cups

The prepared substrates were weighed based on treatments of factor K, then were sterilized with an autoclave at 121 °C for 15 min, then it was cooled to room temperature for approximately 1 h. All utensils used should be cleaned and sterilized as well. 50% flour (80% soybean flour and 20% tempeh flour), 20% tempeh yeast were manually mixed with each mixed substrates. Water was added in a sufficient amount (approximately 90-100 ml) during mixing to hydrate the substrates. Each treatment was cast between two stacked perforated plastic cups, fermented for 72 h in a limited light room, and at room temperature. To stop the fermentation process, the wet-biofoams were dried in an oven at 60^{O}C for 48 h.

Parameters Measurement

Physical Properties

Density (ρ) was measured by dividing the dry mass of the sample in grams (m) by the volume of the foam (v) sample in cm^3 units. Porosity (%) was measured by subtracting the dry foam weighed (m_2) from the wet foam weighed (m_1), and then dividing the result by the volume of foam (length x width x height). Water absorption (%) test conducted by weighing the foam cups and recording the initial weight (m_0). The sample

was soaked in 700 ml of water for 15 minutes and taken out. Then remove the remaining water with facial tissue until no more water drip off from the foam surface, weighed and recorded as wet weight (m_b). The equation of density, porosity, and water absorption is as follows:

$$Density\ (\rho) = \frac{m}{v} \quad (1)$$

$$Porosity = \frac{m_1 - m_2}{volume}\ x\ 100\% \quad (2)$$

$$Water\ absorption = \frac{m_b - m_o}{m_o}\ x\ 100\% \quad (3)$$

Mechanical Properties

The puncture test was conducted using a Brookfield LFRA Texture Analyzer. A sample of 2x2 cm was placed between the clamping plates. The machine was on, calibrated, and the probe was installed, and the load range was arranged as the failure occurred between 10% and 90% of full load, at 300±10 mm/s machine speed. The pressure is intentionally applied until samples collapse. The average given pressure was calculated as a measure of puncture strength. LFRA Texture Analyzer with 18 Probe was used to measure the compressive strength, at a speed of 1 mm/s. The distance between the probe and the material was 0.5 cm. The average and standard deviation of compressive strength were determined from three measurements.

Morphological Properties-Scanning Electron Microscopy (SEM)

The surface of mycelium foam was captured using a SEM-JEOL Type JSN-6510LA. The samples were cut to 1cm x 1cm and taped onto the bronze visualization cross-section. Next, a thin layer of gold was sputtered onto the samples' surfaces at 60 seconds and 20 mA current. A SE detector (Secondary Electron) was used to image the surface of the sample with an electrostatic voltage of 15.0 kV and a width of 11.5-12 mm. The magnification object was 1000x with scaling up to 100μm dan 10μm scales.

Biodegradability Properties

Biodegradability was measured based on the percentage of weight loss of the sample on the soil burial method. The samples were cut into 2.5 x 5 cm and measured the initial weight as w_0, and each sample was covered with gauze and buried in soil. The soil was placed in a plastic bag with a height of 20-22 cm. After 14 days, the samples were dug back and cleaned of soil fragments and measured as final weight (w_1).

Thermal Properties

Thermogravimetric analysis (TGA) is done by using a Mettler TG-50 module attached to a Mettler TC-11 4000 thermal analyzer (USA). Analyses were performed in nitrogen atmospheres with flow rates of 50 milliliters per minute. Temperature settings start from 30 °C to 600^0C with the rate of 20^0C °C/min.

Chemical Properties - Functional Froups Identification

Identification of the functional group was conducted using a Shimadzu FTIR. Each sample was cut into 1 cm x 1 cm and was placed in a holder in the path of the IR source. Analog signals are converted into spectra by the detector. Computers are used to analyze signals and identify peaks. IR beams pass through partially silvered mirrors, which split them into two equal beams.

RESULTS AND DISCUSSION

Physical Properties - Density, Porosity, and Water Absorption

A high-density foam has a compact structure, a low porous surface, and has the ability to prevent water absorption compared to low-density foam. Therefore, the density value is inversely ratio to the porosity percentage of foam. The density of the resulting mycelium foam varied from 0.26 to 0.72 g/cm^{3,} with an average score is 0.43 g/cm^3. Then the porosity percentage of mycelium foam is in the range of 25.93-46.36%, with an average is 35.26%. As shown in the ANOVA, substrate formulation has a very significant influence ($P<0.01$) on density and porosity. As shown both in Fig.-1 and Fig.-2, the density values of foam tend to decrease (Fig.-1) when there is an increasing trend of its porosity percentage increase (Fig.-2) as the percentage of CF increases. Overall, the density of this foam is increased compared to the density of 100% coconut fiber foam, which ranges only 0.08 g/cm^3.[16] Polymeric foams generally have a density between 0.016-0.960 g/cm^3. The density of this resulted mycelium foam is categorized as low-medium density

foams. Medium density foam is used in packaging industries, but its rigidity should be satisfactory to ensure its function as a container.[19] Addition of CP in this research is proven to increase the density and reduce its porosity, produce a more compact structure, and reduce the void in foam matrices. CP contains pectin, which has a monomer of D-galacturonic acid. This acid contains a hydroxyl group that binds to ions Mg^{2+} atau Ca^{2+}, which makes pectin sticky and hydrophobic. These two ions produce an amorphous gel which is expandable in a high moisture environment, especially during fermentation, where the water is trapped within the gel and produces tight covalent bonds even after drying.[20] Vice versa, as the portion of CF increases (samples K3, K4, and K5). Coconut fiber as hydrophillic polysachharides, which absorbed more water during mixing and fermentation time.[21] And after drying, when the water is evaporated, the biofoam matrices with a higher percentage of CF are more porous and less dense.

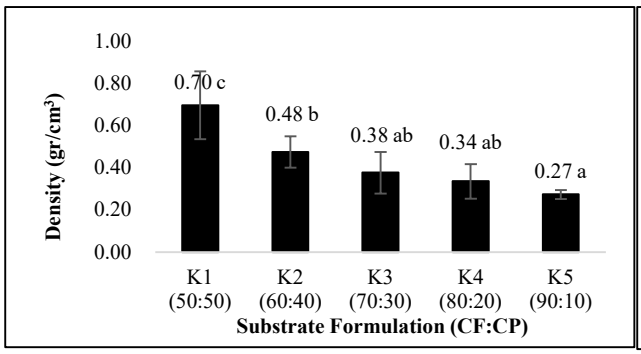

Fig.-1: The Influence of Substrates Formulation on Density of Mycelium Foam ($LSD_{0.05}$ = 0.17).

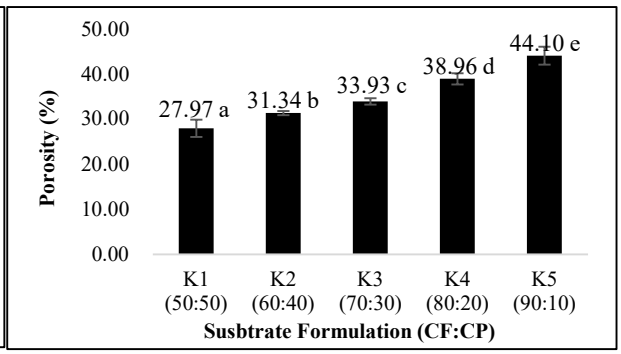

Fig.-2: The Influence of Substrate Formulation on Porosity of Mycelium Foam ($LSD_{0.05}$ = 2.54)

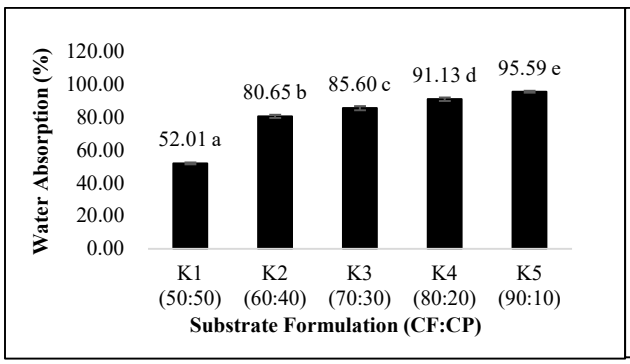

Fig.-3: The Influence of Substrates Formulation on Water Absorption of Mycellium Foam ($LSD_{0.05}$ = 1.71)

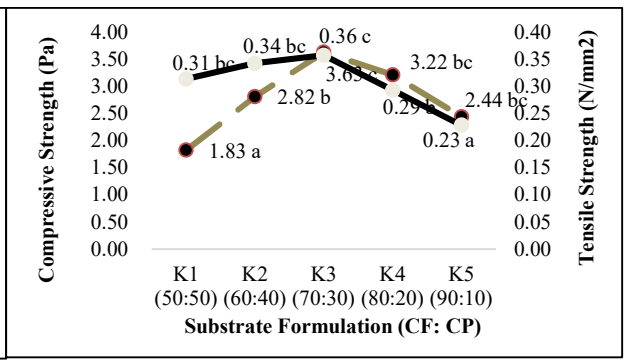

Fig.-4: The Influence of Substrates Formulation on Compressive Strength ($LSD_{0.05}$ = 0.52) and Tensile Strength ($LSD_{0.05}$ = 0.05) of Mycellium Foam

The foam has water absorption in the range of 51.43%-96.25%, with an average is 81.00%. ANOVA suggests that substrate formulation significantly influences ($P \leq 0.01$) water absorption. Figure-3 shows the linear relationship between the CF increasing or CP decreasing portion in substrate formulation to water absorption rate. K1 has lower water absorption, since it has lower portion of CP than others. CP has pectin, the hydrophobic molecules which are impermeable to water in biofoam. On the other hand, K3, K4 and K5 have higher water absorption rate. CF contains cellulose and hemicellulose, the hydrophilic molecules and produces more porous matrix, which is also absorbs more water.[22] CF in this research utlizes coconut husk, which has suberin, an impermeable lipid wax which is supposed to improve the water permeability of the foam.[23] However, utilisation of CH in this research might be not yet sufficient since the water absorption is slightly improved from previous research. Previous research stated that a biofoam cup with 100% CF undergoes the tempeh fermentation for 3-5 days, has a water absorption range from 78.00% to 97.00%[24], which is higher than this research.

Mechanical Properties - Puncture Test and Compressive Strength

The puncture test of mycelium foam varied from 0.20–0.37 Nmm^{-2}, and the average is 0.31 Nmm^{-2}. The compressive strength ranges from 2.00–3.73 KPa, with an average is 3.13 KPa. ANOVA shows that

substrate formulation has a very significant effect (P≤0.01) for both parameters. K3 foam has the highest compressive strength and puncture test, which is also significantly different from other treatments. Commonly, the compressive strength is correlated with matrix density and its compactness.[25] But this finding is quietly different from other previous research. K1 is reported to have a more compact and dense matrix, but its mechanical properties are lower than K3. K1 has a brittle texture and presents an uneven surface. Foam with a huge void is easy to collapse when pressure is applied, since the void leads to the presence of small pores.[26]

Morphological Properties – Scanning Electron Microscopy

Figure-5 shows the look of the resulting mycelium foam. This foam has a smooth surface with white yellowish, a specific colour of tempeh yeast, and the entire substrate is covered by mycelium. This is because mycelium grows on both sides of a surface, inside the cup, and builds up the mycelium as a colony formation. Tempeh yeast extracted chitin for outer surface and as matrix adhesion, and β-glucan for inside the cell walls. As the cell walls form a network, hyphae are built up, fill up the void and strengthen the bond, and make mycelium. Morphological properties with SEM of all five samples based on substrate formulation levels could be seen in Fig.-6.

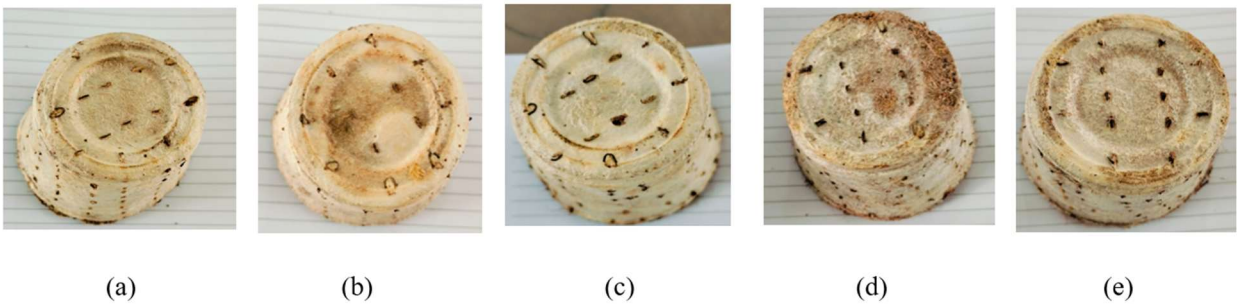

Fig.-5: Mhycelium Foam Samples Based on Substrate Formulation (CF: CP) (a) K1 (50:50); (b) K2 (60:40); (c) K3 (70:30); (d) K4 (80:20), K5 (90:10)

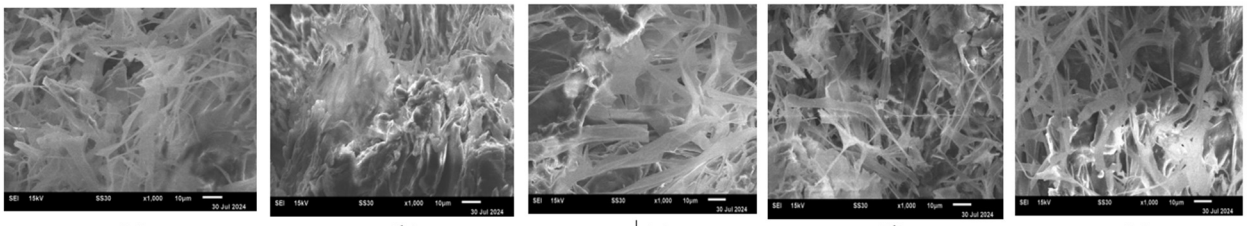

Fig.-6: Scanning Electron Microscopy (SEM) of Five Mycelium Foam Samples Based on Substrate Formulation (CF: CP) (a) K1 (50:50); (b) K2 (60:40); (c) K3 (70:30); (d) K4 (80:20)) K5 (90:10)

Figure-6a and 6b show structure foams of K1 and K2 are dense, the mycelium looks tight with thin hyphae stacked in the form of fibers, although CF is still seen as a substrate for fungal growth. The thin hyphae are suspected to affect the low compressive strength and tensile strength of the samples K1 and K2. In K3, K4, and K5, as seen in Fig.-6c, 6d, and 6e, morphological texture looks more dense than in K1 and K2. All figures show mycelium is bonded and connected, and forms a compact matrix. The long-tubular hyphae with varied diameters are formed and appear side by side with the thin fiber hyphae. Even several tubular hyphaes seem to stand alone and are not bonding with each other. The mycelium foam of K4 and K5 matrix looks more porous. The porous looks might be a reason why the compressive strength and tensile strength of K4 and K5 are lower, but the water absorption is higher.

Biodegradability Properties

Packaging with natural polymer is estimated to degrade in 60 days. [27] The rate of degradation of mycelium foam in the soil burial method varied from 18.67%-58.30%, with an average score of 36.60%. ANOVA

suggests that substrate formulation has a very significant influence ($P \leq 0.01$) on the degradation rate of mycelia foam, as can be seen in Fig.-7.

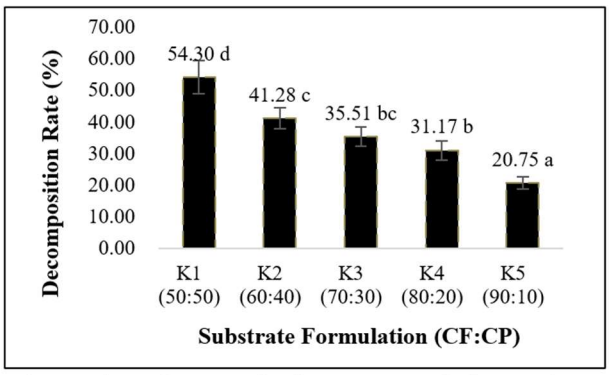

Fig.-7: The Influence of Substrate Formulation on the Decomposition Rate of Mycelium Foam ($LSD_{0.05}$ = 6.56)

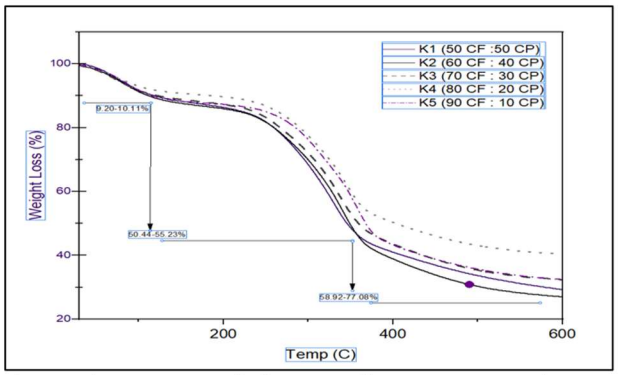

Fig.-8: Thermogram of Five Mycelium Foam Samples Based on Substrate Formulation

Biodegradability is influenced by many factors such as the thickness, type of substrates, and substrate composition. In Fig.-7 shows that K1 presents the ability to decompose quicker than other treatment levels. CP contains polysaccharides such as cellulose, pectin, hemicellulose, nitrogenous compounds such as caffeine and trigonelline, and a smaller amount of lipid.[28] Caffeine, trigonelline, and lipid available in coffee pulp combine with the soybean and tempeh flour tend to promote enzymatic hydrolysis and accelerate the degradation to microphase through microorganism activities in the first week soil burial test.[29] The residual sample after the soil burial test mostly consists of CF, and the residual weight increases as the CF percentage in the substrate formulation increases. CF mostly contains 43.44% cellulose and 45.84% lignin. Cellulose is composed of glucose, which is linked with beta 1,4 glycosidic linkages, which are more resistant to acid hydrolysis and make cellulose difficult to degrade. The beta-glycosidic linkages enable the cellulose and hemicellulose to form crystallites in a very dense structure.[30] This dense structure prevents water or enzymes from penetrating and breaking up the bonds.

Thermal Stability

The TGA curve in Fig.-8 provides information about the weight loss during the increasing temperature. [31] In phase I, an endothermic process takes place until the temperature reaches 100.00 °C. The water and bonding water inside the matrix are evaporated, and the weight loss of foam in phase I is around 0.05-0.14 mg or 9.20-10.11% with a heating temperature around 150 °C. Then stage II - exothermic stage, degradation starts, and shows the foam heat-retaining figures. The thermal degradation temperature of K1 started at 302.41 ºC and subsequently increased to 314.27 ºC-320.63 ºC for other treatments. These ranges are similar to the thermal degradation temperature of the biofoam cup from 100% coconut fiber. It can be seen that the thermal degradation temperature of the five samples tends to increase as the percentage of CF increases. This might occur since CP has a lower thermal degradation temperature than CF. Cellulose in CF requires a higher temperature to break down the beta-glycosidic linkages and converts the free glucose to CO_2, H_2O, and other kinds of hydrocarbon products, such as volatile compounds. Meanwhile, hemicellulose and lignin are compounds that will increase the carbonation residue. Then at temperature reaches 400 ºC – 600 ºC stage III is started. At this stage, all the residues are burnt and resulting in ash.[32]

Chemical Properties-Functional Groups

Figure-9 shows that two out of five biofoam samples present the absorbance character for polysaccharides, alkyl groups (C-H), K1 at 2910 $cm^{-1,}$ and K2 at 2995 cm^{-1}. Alkyl groups recorded at 2970-2860 cm^{-1} [33], alkyl is an unstable group and is formed by the removal of a hydrogen from a carbon group, such as methane, and generates a methyl group. The presence of alkyl groups is only identified in K1 and K2 since these have a higher percentage of CP. A hydroxyl (O-H) groups are identified at 3269 cm^{-1} for K3, at 3277 cm^{-1} for K4, and at 3280 cm^{-1} for K5, classified as normal polymeric OH stretch.[33] Hydroxyl groups commonly appear at 3570-3200 cm^{-1}.This result is not much different from the study of biofoam from 100% CF with wave wavelength of hydroxyl groups at 3273.20 cm^{-1}.[24] In K3, K4, and K5, the presence of amines

or amide groups (N-H), triple bonds carbon or triple bonds carbon with nitrogen at wavelength 3400-3380cm^{-1}. In all five samples, ethers and carbonyl groups (C-O) and alkenes (C-C) are found in wave length 1200-1070 cm^{-1} for ethers with C-O stretch, 1800-1680 cm^{-1} for carbonyls with C-O stretch. Carbonyls, ethers, amines, and amide groups could be presented as flavonoid and aromatic compounds from fermentation, such as ethanol, ester, propionic, mixed-acid, butyrate-butanol.[34]

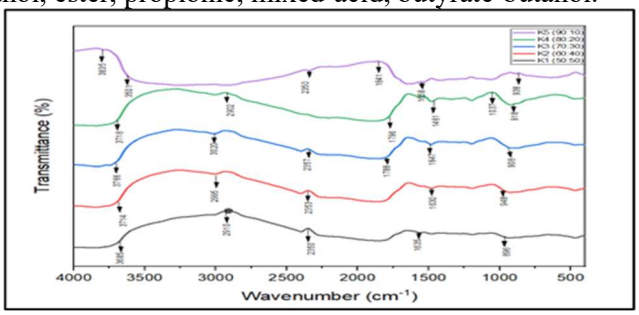

Fig.-9: Transmittance and Wave Number of Five Mycelium foam Samples Based on Substrate Formulation (CF: CP) (a) K1 (50:50); (b) K2 (60:40); (c) K3 (70:30); (d) K4 (80:20)) K5 (90:10)

CONCLUSION

Composites of CF and CP have huge potential to be used as a substrate for fungal mycelium growth in the production of 100% eco-friendly biofoam. As a higher percentage of coconut fiber, up to 90%, used in substrate formulation, the water absorption, porosity, and thermal degradation temperature increase, slow biodegradability in soil burial test, and less weight loss in thermal degradation are also obtained. Meanwhile, the usage of coffee pulp, up to 50%, tends to produce biofoam with higher density, lower porosity, and thermal degradation. Optimization should be achieved by adjusting the substrate formulation to 60-70% coconut fiber and 30-40% coffee pulp, and adding a thin layer of edible biofilm on the biofoam surface to produce optimum quality biofoam.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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