Experimentation and Characterization of Mycelium-Based Biocomposites for Ephemeral Product Applications

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Abstract. Single-use plastics, due to their ephemeral nature, are a problem for sustainability. To overcome this difficulty, biomaterials are being created. A biocomposite based on mycelium and six different substrates has been developed to study its characteristics and possible applications in the substitution of ephemeral plastic-based products. The use of six leftovers or biological waste allows results to be compared between samples, to determine which leftovers or waste are revalued instead of being eliminated, in accordance with the principles of the circular economy (CE). Samples and specimens were developed for laboratory tests to characterize density, water absorption, and compression. All tests were carried out according to norms and standards that correspond to the plastics they can replace. Results indicate that the 6 types have very low densities, even lower than polymeric foams. They present good substrate properties in terms of compressive strength, with values similar to expanded polystyrene (EPS). In addition, all the samples are biodegradable since they do not require any type of coating, they can take a wide variety of shapes and the molds can be developed using various manufacturing techniques. Potential applications are found in the packaging industry since ephemeral containers require a certain resistance and low weight, being able to replace EPS or alveolar film. Being an inert material, it could be used in food packaging and even replace some applications of cardboard or paper pulp.

Keywords: Biocomposites · Mycelium · Ephemeral products · Sustainability · Eco-design

1 Introduction

Currently, one of the main obstacles to sustainability is the indiscriminate use of single-use plastics, they are ephemeral due to their limited use and its very short life cycle [1]. A high percentage of garbage are plastics, and most of them come from single-use containers and all kinds of wrapping and packaging [2]. The European Union in July 2021 banned the use of some single-use plastic items [3], this law includes the prohibition

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S. Gerbino et al. (Eds.): JCM 2022, LNME, pp. 15–27, 2023.
https://doi.org/10.1007/978-3-031-15928-2_2

of containers and cups for food and beverages made of EPS, including their lids and plugs.

Another alternative to the prohibition of the use of this type of plastic is recycling, although it has various disadvantages. According to Ecoembes [4], one in four of the waste in the yellow container was not correctly located. Also, plastic cannot be recycled an infinite number of times, as the molecular chains are degraded. For these reasons, some types of plastics cause a serious environmental problem that must be solved through the exploration of other alternatives. One of the main plastics in landfills is EPS, with good qualities such as versatility and ease of shaping, shock absorption or lightness. In addition, EPS has very good mechanical resistance, and is a good insulator against cold and heat [5]. There are already studies on the substitution of these polluting materials in containers of various types and studies on the characteristics of the substitute materials of an organic nature [6–9].

The increased demand for green materials has given rise to a large number of studies on biocomposites [10, 11]. Biocomposites are defined as composite materials where biopolymers form the matrix and are reinforced by natural fibers, where the fibers are usually of organic origin. A very important factor of biocomposites is the possibility of taking advantage of biological remains or residues such as shells, fibers or residual stems. In this way, the remains or waste are recovered instead of being discarded, in accordance with the principles of the CE [12]. Circular economy is defined as a production and consumption process that involves sharing, renting, reusing, repairing, renewing and recycling existing materials and products as many times as possible to create added value, so the life cycle is extended. It implies reducing waste to a minimum and for this reason the promotion of this type of material favors this process [13].

This study presents the production and characterization process of a biocomposite, as well as tests performed in biology laboratory from LIA-CESAR laboratories of the University of Zaragoza at the Etopia Center for Art and Technology. The aim of the study is to design and experiment with a new biocomposite that can replace in some applications plastic materials with strong environmental impact. Mycelium-based biocomposites contribute to the principles of the CE since hardly any new raw materials are used, the production process requires a low energy input and they are biodegradable. To achieve this, the material is characterized by means of mechanical tests.

The expected result is a new material tested with six different fibers, and the definition of its characteristics and potential applications in the design of environmentally friendly products. Another objective is to determine which organic waste has better behavior. After analyzing the results, their characteristics are found to be similar to those of single-use plastics, thus being suitable for applications of ephemeral products that can be replaced to approach the CE.

2 Materials and Methods

New materials that respect the environment are emerging, some of them being biocomposites made from mycelium, the vegetative body of a fungus. Mycelium-based biocomposites use natural organism as the fungus for the matrix, organic recycled materials for the filler material or reinforcement, the production process requires low energy input and they are biodegradable being an ideal compound for the CE. In the scientific literature, some works studied characteristics such as density, thermal conductivity, Young's modulus, compressive strength, flexural strength, tensile strength, response to exposure to moisture or response to immersion in water, among others [10, 14, 15]. The culture of the mycelium has also been studied to refine and achieve the best results [16, 17], including engineering, architecture and design applications [16, 18], or articles already marketed for own crops [19], consumer products [20] or registered materials as *Mycocomposite* [19], *Mylo* [21] or *Fungicel* [22].

The methodology followed during this process starts with the production of six biocomposites resulting from the growth of *Ganoderma Lucidum* fungus on a filler material that in all of them is an organic substrate, these are: (a) wood chips, (b) straw, (c) pellets, (d) cotton from old T-shirts, (e) peanut shell and (f) cardboard. The objective is to submit each of the composite materials to three physical tests: compressive strength, hardness and water absorption, the density will also be determined. With the data obtained, their properties are known and comparisons can be made between them and the plastic materials to be replaced.

2.1 Regulations, Tests and Specimens

Prior to the growth process of the material, the tests to which it will be subjected are defined. Due to the fact that there is no specific regulation, the same tests to which polymeric foams such as EPS are subjected are carried out. These tests will be: compressive strength, hardness and water absorption, including the calculation of density. The specimens of the material that will be used in the three tests are created, with a shape conditioned by the dimensions of the laboratory machines for the tests.

To define a specific geometry, the standard must be consulted. "Rigid cellular plastics - Determination of compression properties" version ISO 844:2021 and the Spanish version UNE-EN ISO 844. According to the standard, the specimens must have a thickness of (50 ± 1) mm and that the base must be either square or circular, with a minimum area of 25 mm^2 and a maximum of 230 mm^2. The plate of the compression machine is 45 mm in diameter. It is determined that the specimens should be cylindrical with a base of 45 mm in diameter and 50 mm in height.

Before the start of the project, a first version of the mold is made in the fabrication laboratory of LIA-CESAR in Etopia. In this mold, made by 3D printing, the biocomposite specimens will be grown. These first samples are subjected to compressive strength and hardness tests to check if the selected dimensions are correct. Once the dimensions of the specimens are verified, a final version of the mold is made.

2.2 Culture and Growth of the Specimens

For the growth phase, it is necessary for the biocomposite to have 80% cellulosic sugars and 20% proteins and approximately twice the weight of water is added to the mixture (Table 1). Sugars are provided by organic lignocellulosic residues and proteins are added thanks to parts of bran, brown rice flour or coffee waste. Three samples for each substrate are grown as experimental replicates to obtain more accurate results in physical tests. It should be noted that in several substrates the protein source is a wholemeal flour (called BRF, brown rice flour). In Table 1, the proportions of matter for each substrate are shown. Figure 1 and Table 2 shows the cultivation and growth process.

Table 1. Proportion of water in the substrates

Sugars (80%)	Proteins (20%)	Water
10 g wood chips and 10 g wood dust	2,5 g bran and 2,5 g de BRF	50 g
10 g straw and 10 g wood dust	2,5 g bran and 2,5 g de BRF	50 g
20 g Cotton fibers	2,5 g bran and 2,5 g de BRF	50 g
20 g cardboard	5 g grounded coffee	60 g
40 g pellet	10 g grounded coffee	150 g
25 g peanut shell	25 g	

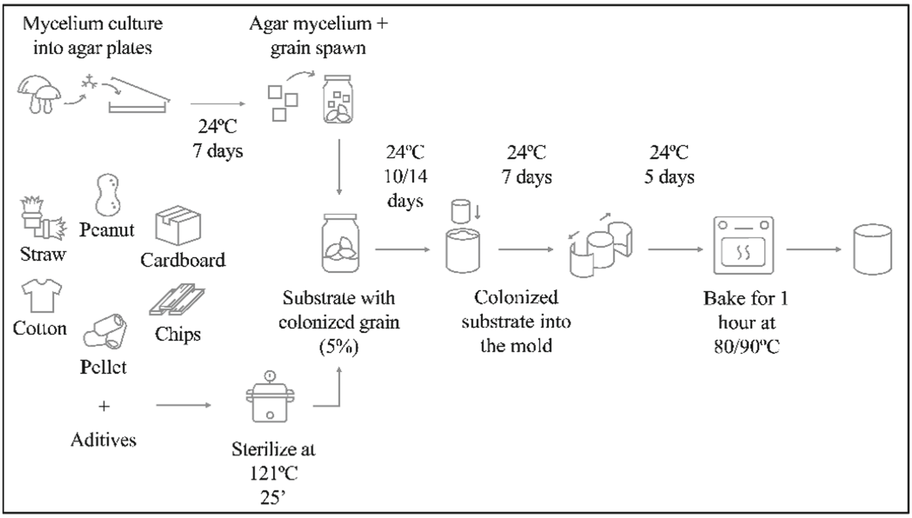

Fig. 1. Cultivating and growing process of mycelium-based composite. Based on [10]

Table 2. Cultivating and growing process of mycelium-based composite

Stage	Actions
1. Preparation plates with Agar Fungi	Substrate: 2.3 g Agar; 4 g malt extract; 0.4 g yeast extract; 0.4 g Peptone Boil the mixture. Pour into a petri dish
2. Reproduction of the fungus in Agar	Fungi Selection of *Ganoderma Lucidum*. Place mycelium of the fungus in the Petri dish. Introduce in the incubator at 22–25 ºC
3. Preparation of the wheat grain	Wash and hydrate the wheat grains for 24 h. Boil grains 15′. Sterilize the grains in the autoclave
4. Colonization of wheat grain	Cut 1 cm^2 squares of *Ganoderma Lucidum* agar plate culture. Put 3 or 4 squares in the grain container and shake. Incubate for 4 to 6 days at 22 ºC for the mycelium to colonize the grain
5. Preparation of the substrates	Cut the substrates in a glass blender. Mix the cut substrates with water. Pour the mixture into a container and sterilize by autoclaving at 121 ºC 25′
6. Mix substrate with fungus	Introduce the colonized grain (5%) into the jars with the substrate. Incubate from 10 to 14 days (depending on the substrate) at 22 ºC
7. Shape the material	Crumble the colonized substrate in a tray. Introduce the colonized substrate into the mold pressing it with a piece of wood. Incubate for 6 to 8 days (depending on the substrate) at 22 ºC. Put the mold in a plastic zip-lock bag slightly open at one end to keep humidity letting air flow. Incubate outside the mold for about 5 days
8. Bake and dry	Bake for 1 h at 80/90 ºC (take care not to burn). Finish drying outside for a further 7 days

2.3 Description of the Tests

Water Absorption

To carry out this test, the standards ISO 15148:2002 "Hygrothermal performance of building materials and products - determination of water absorption coefficient by partial immersion" and ISO 15148:2002/AMD 1:2016 "Hygrothermal performance of building materials and products - determination of water absorption coefficient by partial immersion - Amendment 1" have been followed. As the norm says, a metal grid is placed in a plastic container, the grid is always parallel to the base and at the same distance from the bottom. The level at which the water should be is 5 mm from the highest point of the grid. Fill the container with water up to the indicated limit and place the 6 samples on the grid. A strip of wood is placed over the samples to prevent movement as the samples could float on the water.

Eight measurements are taken over 24 h. These measurements will be after 5, 20 min, 1, 2, 5, 8 and 24 h from the moment the samples are placed on the grid. The test gives rise to graphs (Fig. 1) where the amount of water absorbed by the material is observed as the test progresses and this is carried out by facing the following parameters: ΔMt (kg/m^2) Difference between the mass in each weighing; and, $\sqrt{t}$ (s) Square root of the times in which each weighing is performed.

Compressive Strength

For the compression test the Instron 5565 machine is used. To carry out this test, the standard ISO 844:2021 "Rigid cellular plastics - Determination of compression properties". According to the experience of the prior tests, the machine is programmed to interrupt the test when it reaches a load of 4000 N. Although the previous test was carried out only with Chip samples, it was observed that around this value the samples stopped reducing their height. The speed has been 5 mm/min, the UNE-EN ISO 844 standard which says that: "the sample is compressed at a speed as close as possible to 10% of its original thickness per minute". The sample is 50 mm high, so the speed is 5 mm/min, it was found that at that speed the sample had optimal behavior in the steps that took place throughout the test.

According to the standard, the material can behave in two ways. Mode A: the specimen reaches maximum force and breaks. Mode B: There is no maximum point and the specimen is compressed without breaking. Some specimens have behavior A and others B. The standard establishes some parameters to be calculated depending on whether one behavior or another. In order to compare the test results for each material and follow a single criterion, it is established that the parameters corresponding to Mode B will be calculated. According to Mode B, the parameters to be calculated according to the standard are: Compression stress at 10% of its relative deformation σ (Compression strength MPa); and, Modulus of elasticity or Young's Modulus E (MPa).

Hardness

To carry out the hardness test, an analog Shore A scale hardness tester is used, since this is the one used for soft polymeric materials. To carry it out we take from 6 to 9 measurements depending on the material of the sample. The measurements are taken on the flat faces and depending on the substrate with which they have been made, they have a more or less uniform surface, so it is convenient to take many measurements if it is irregular. The final hardness result is the average of the measurements obtained.

The hardness test is carried out again after the compression test to check how the hardness changes when subjected to a load, in this case 4000 N. In this way, possible properties and applications can be seen if it is subjected to compression processes.

3 Results

The 6 biocomposites present average density values of between 0.19 and 0.37 g/cm^3, lower than EPS. This plastic can have values between 0.02 to 0.31 g/cm^3 or even higher depending on the application, which places the biocomposite, within the range, so it can replace EPS in applications where low density is required (Table 3).

Table 3. Mean values for density

Sample	(a) Chips	(b) Straw	(c) Pellet	(d) Cotton	(e) Peanut	(f) Cardboard	EPS 50	EPS 250
Density (g/cm^3)	0,23	0,19	0,30	0,37	0,27	0,37	0,02	0,31

The first test is the water absorption test as this is not destructive. The calculation of the density of each sample will be carried out at the same time as the last two tests to ensure that the dimensions of the sample will not be altered further due to the stabilization of the weight. For water absorption the results show that all substrates behave differently, as seen in Fig. 2. Both absorption coefficients are compared with the density of the samples to check if there is a relationship between both characteristics. It can be said that denser substrates tend to absorb more water.

In the compression test (Tables 4 and 5), the samples show two different behaviors. Pellet, peanut and cardboard based samples disaggregate or break, the test is interrupted before reaching 4000 N of load. Both pellets and peanuts disaggregate whereas the cardboard substrate compresses without disaggregating, but cracks inside. Other samples are compressed without breaking, wood chips, straw and cotton support the 4000 N load (Fig. 3). The cotton substrate is the one with the highest 10% compression stress (0.38 MPa). The hardness test was carried out on the specimens both before and after submitting them to the compression test. This allows us to check how this characteristic varies. The Shore A value ranges from 43 for straw to 68 for cardboard. Relating hardness and density, it can be seen that, the higher the density, the samples have greater hardness.

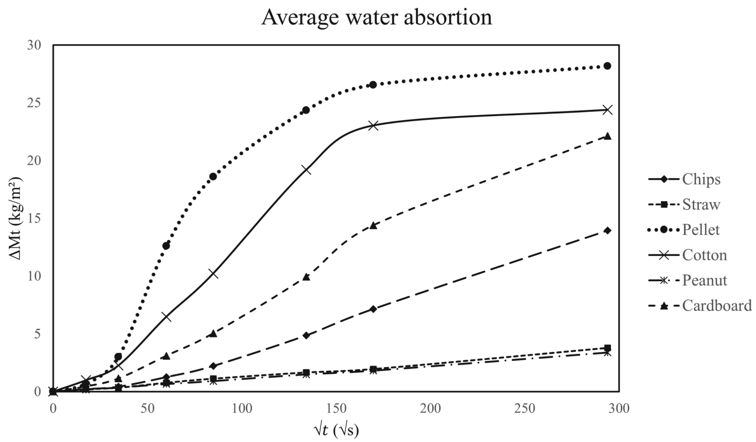

Fig. 2. Average water absorption (over 1 day)

The graphical and numerical results of the compressive strength test are shown, in Figs. 4 and 5. The results correspond to the average of the samples for each filler material.

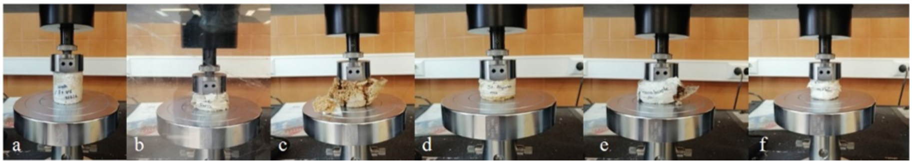

Fig. 3. Images of the tests of the different samples

Table 4. Compression resistance test

Filler material	Behavior	Pass
(a) Chips	Compressed sample, but without presenting any internal rupture. Only the outer faces are slightly cracked	Yes
(b) Straw	Compressed sample, but without presenting any internal rupture. Slightly cracked outer faces. The final result is very similar to that of straw, very similar substrates	Yes
(c) Pellet	The test is interrupted before reaching 4000 N the sample breaks and disaggregates	No
(d) Cotton	Very good behavior. It decreases its height but without suffering breaks, neither internal nor on the external faces	Yes
(e) Peanut	Good compression behavior in the first moments of the test. It eventually cracks and breaks	Yes
(f) Cardboard	Internal break before the end of the test, but the sample is hard and solid and does not show breaks on the external faces	No

Table 5. Values resulting from the compression test

	(a) Chips	(b) Straw	(c) Pellet	(d) Cotton	(e) Peanut	(f) Cardboard
Compressive strength (MPa)	0,11	0,08	0,13	0,38	0,17	0,13
Modulus of elasticity (MPa)	1,76	1,16	2,09	2,71	2,17	1,81

4 Discussion

The low density of this mycelium-based biomaterial is one of its most attractive aspects. However, this characteristic cannot be finely controlled in the growth process of the material and varies significantly in the phase in which it is introduced into the mold. Being a manual process, it varies between one test to another and in the case of taking it to industrialization it would be necessary to carry out an ad hoc quality control. This characteristic is very favorable for the manufacture of packaging products, which must be as less dense as possible. Taking into account that the lower the density, the lower the compressive strength, these products must to ensure that both properties tend to balance these values, packaging must be light but resistant to shocks.

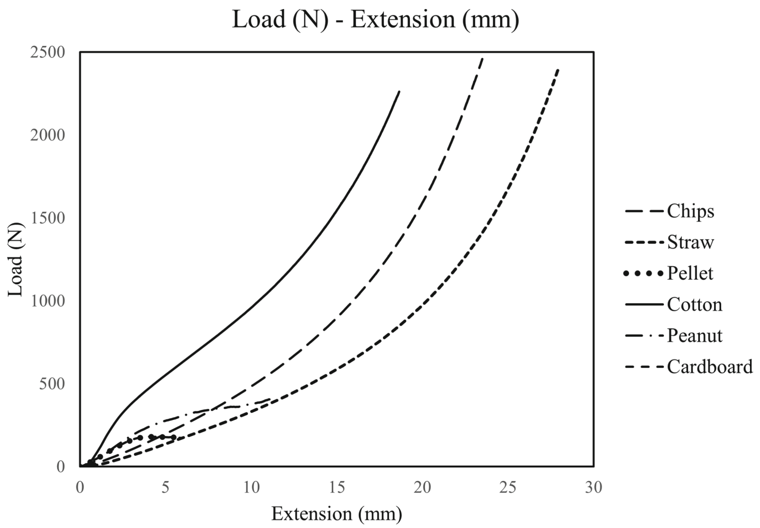

Fig. 4. Extension in the compression test

From the Stress (MPa) - Average Strain graph (Fig. 4) the following conclusions can be drawn. The samples present two different behaviors, as stated in the standard. Some disaggregate, in which case the test must be interrupted in the case of pellets and peanuts, while other samples are compressed without breaking and the graph does not reach a maximum. The cotton substrate is the one that presents a compressive stress at 10% higher (0.38 MPa) with no breaks. The chips and straw substrates do not break either. Cardboard substrate, is compressed without disaggregating but it cracks inside. Therefore, when choosing one substrate, it is also possible to consider whether the part made with the biocomposite must be sacrificed or its integrity must be maintained.

Although cotton has also the highest modulus of elasticity, so do granules and peanuts. Thus, it is necessary to apply a higher tension than in the rest of them to undergo the same deformation. When comparing density and compressive strength, the graph (Fig. 5) shows that the substrates follow a slight trend, the lower the density, the lower the compressive stress and vice versa. Regarding the relationship between density and elasticity modulus, they show a similar behavior with the exception of the cardboard and pellet. The lower the density, the lower the modulus of elasticity and vice versa.

Cotton substrate appears to be a good material to replace some EPS parts due to its high compressive strength relative to its low density. This could be because the fibers are small pieces of tissue that, despite being mixed with water, do not break and, by joining the mycelium, create a resistant mesh. On the other hand, chips and straw are also compressed without breaking, becoming a more compact material because the fibers of these substrates are small but elongated. In the material preparation phase, in which they are mixed with water, they do not fall apart and maintain their integrity so that,

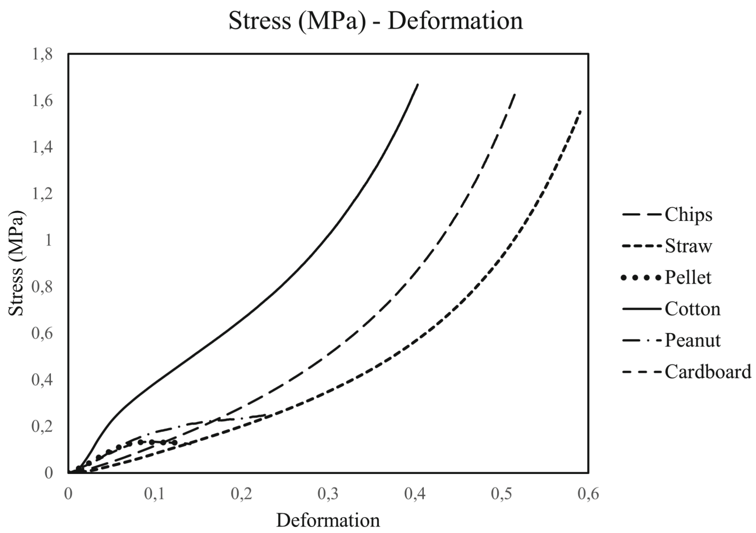

Fig. 5. Deformation in the compression test

together with the mycelium, create a material that is compressed but does not break. On the other hand, the cardboard samples have larger fibers than the rest, but when they get wet they dissolve, which can influence their compressive strength. And finally, the test peanuts and granules are broken up and disaggregated. The fibers of the latter are short and do not facilitate union with the mycelium, being less resistant and durable. For the manufacture of containers with mycelium, the ideal properties are high resistance to compression and low density. The density slightly affects the compressive strength, the higher the density, the greater the resistance (Fig. 6).

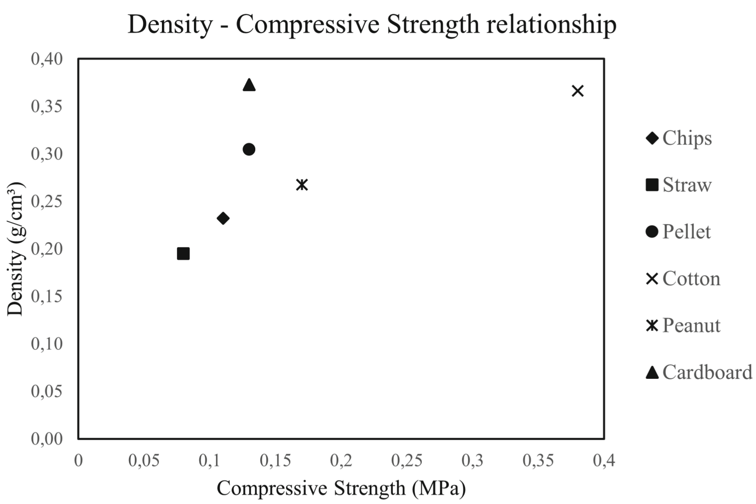

Fig. 6. Relationship between density and compressive strength

The type of fibers of each substrate also influences. The best option is a fabric such as cotton, since its intertwined fibers provide great resistance, although it is the densest sample. This property can be controlled during the growth phase of the material being molded. By reducing the pressure when the material is fed into the mold, the density can be reduced. It would be necessary to verify if this variation in the manufacture of the material does not worsen the properties in terms of compressive strength. On the other hand, although chips and straw have lower compressive strength values, their low density makes the balance between these two properties ideal for the manufacture of some products. This shows that the elongated and fine fibers provide good properties to this compound.

Some properties of the developed samples should be tested in future works, since mycelial materials possess certain fire retardant properties and could be used as an economical, sustainable and fire safer alternative to synthetic polymers [23]. Electromagnetic microscope images will help to understand the growth to select the best substrates.

5 Conclusions

The 6 types of the mycelium biomaterial have a very low density, even lower than polymeric foams. They have good properties against resistance to compression, placing some substrates with values similar to EPS. All samples are biodegradable since no coating is applied, can take a wide variety of shapes and molds can be developed using various manufacturing techniques.

The behavior in the tests is conditioned by the type of fibers of each substrate. In terms of resistance to compression, the cotton substrate is the one that has the best behavior since its fibers start from a previously woven material, which makes it very resistant. Those with the smallest fibers break and therefore disaggregate.

The density of the samples influences their hardness. Those that are denser have higher hardness values, and vice versa. This is because porosity causes the durometer to encounter less resistance in the test. It can be seen how by compressing the material and reducing the air inside it, the hardness of the samples increases.

The density of the samples is not significantly related to the water absorption coefficients. It seems that it has more to do with the water absorption capacity of the substrate itself. For samples to have higher hardness values, the density must increase. So that the products made with this material do not suffer plastic deformations on their surface, a balance must be sought between density, compressive strength and hardness. On the other hand, it is sought that the material has low water absorption coefficients so that it is stable in high humidity working conditions.

For all these reasons mentioned above, we can conclude that mycelium-based bio-composites have the potential to be applied in containers and packaging that require a certain resistance and low weight to replace EPS or other plastic foams. In addition, since these bio-composites are inert materials, they could be used in food packaging and even replace some applications of paper or cardboard pulp used in electrical or electronic devices.

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