

## Synthesis of a bioplastic from agri-food waste

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**Abstract:** The present dissertation aimed to develop a bioplastic from agri-food waste, namely insect exuviae. The extracted component, chitin, was transformed into chitosan, which is mostly used to produce bioplastics. The extraction of chitin was accomplished by demineralization followed by deproteinization and discolouration. Its transformation into chitosan was achieved by deacetylation. A degree of deacetylation of 71.3 %, was obtained which is higher than the required (60 %) to be considered chitosan. All products were fully characterized by scanning electron microscopy (SEM/EDS), Fourier-transform infrared spectroscopy (FTIR) and thermal gravimetric analysis (TGA).

To produce bioplastic, in an aqueous media, glycerol was used as dispersant, gelatine or agar as a binder. The chitosan and acetic acid (used to improve chitosan solubility) ratio were tuned to obtain the intended consistency. Several samples were produced and the selected ones were PG33 (using agar and chitosan) and PG38 (using gelatine and chitosan) were tested for mechanical behaviour. The first formed a thin film, very malleable, whereas the second was thicker, but still malleable. The strain values obtained were 0.01 and 0.39 N/mm<sup>2</sup>, respectively.

In addition, a successful scaling up, at bench-scale, of the process was achieved. The produced bioplastic at a larger scale was characterized by FTIR and TGA and proven to be identical to the previously obtained. This is a major step in the process, since it represents a potential method to produce sustainable bioplastics.

**Keywords:** Bioplastic, Agri-food waste, Chitin, Chitosan

### 1. Introduction

Nowadays, plastics play a big role in making our modern society easier. Plastics are attractive due to their low cost, high durability, flexibility, lightweight, etc., and are used in different areas, from agriculture to packaging and automotive (figure 1). The production of plastic is essential to our society and it does not show any signs of decreasing.

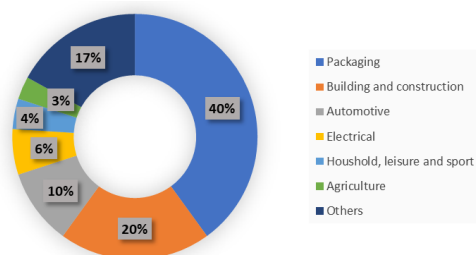


Figure 1 – Uses of plastics. Adapted from [1].

Although plastics help to create a sustainable society and develop technology with more efficiency, the excessive use of plastic has triggered environmental challenges, like the greenhouse gases emissions. It also led to the

contamination of ecosystems, including human health, with micro- and nano-plastics. [1,3]

Therefore, it is necessary to find new ways to replace petroleum-based polymers by biodegradable bioplastics. [2,3]

This work aimed to develop a new type of bio-based plastic from a biomass source. The bioplastic was produced using an insect, the black soldier fly exuviae, which is a bioindustry by-product, through laboratory treatments and was chemical, physical and mechanically characterized. Although only produced at a bench-scale it presents the required properties to allow its production to be up-scaled, thus being promising as a material that benefits the environment, by reducing the carbon footprint and improving the ecosystems.

### 2. Overview of bioplastics

#### 2.1. Definition of bioplastics

Plastics consist of a polymer, that can have additives, such as stabilizers, plasticizers or flame retardants. [1]

Bioplastics can be defined as a plastic-like material that is bio-based, biodegradable or both. Figure 2 classifies the most common types of plastics according to their biodegradability and bio-based content.

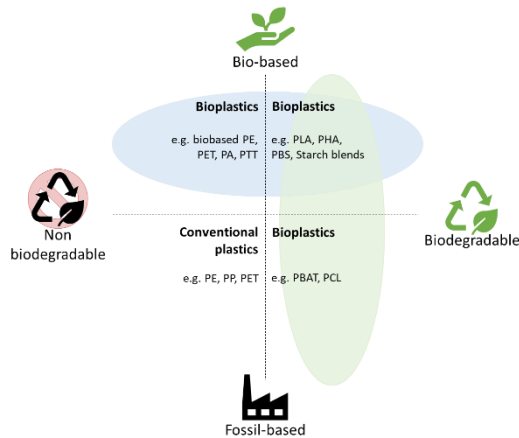


Figure 2 – Material coordinate system of bioplastic. Adapted from [4].

When compared with the traditional fossil-based plastics, the bioplastics have a lower carbon footprint. On the other hand, bioplastics have more waste management options, like organic recycling and recovery of the product at its end of life.

### 2.1.1. Bioplastic market

Nowadays, society aims to decrease the environmental impact of plastics and industries have started to research, develop and produce bioplastics.

The demand for bioplastics is increasing, leading to the growth and diversification of the bioplastics market. In 2019, biodegradable bioplastics represented 55.5 % of the global production capacity, whereas, mixed plastics represented 44.5 % [1, 5] (figure 3).

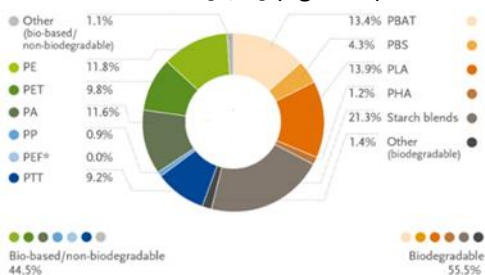


Figure 3 – Global production capacities of bioplastics 2019. Adapted from [1, 5].

## 2.2. Chitin

Chitin (figure 4) is a natural acetylated polysaccharide formed by N-acetyl-D-glucosamine groups that are linked by  $\beta(1-4)$  linkage. [8]

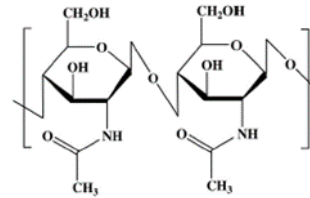


Figure 4 – Chemical structure of chitin. Adapted from [9].

Chitin is the second most abundant resource in nature (being the first cellulose). This polysaccharide is mainly found in crustacean and insects exoskeletons, but also in the cells' walls of fungi and microorganisms. The major sources of commercial chitin are shrimp, crab, lobster, prawn and krill shells. [6, 7]

Chitin is very attractive for various applications in biomedical, food, chemical and other industrial fields. It is biodegradable, biocompatible, non-toxic, eco-safe, renewable and abundant. It is also preferred to synthetic polymers, because is cheaper and present in natural living organisms. [6, 7]

Only 20-30 % of the crustacean's exoskeleton is chitin. Therefore, it is necessary to extract and isolate chitin. Usually, this process needs three consecutive steps: i) demineralization, ii) deproteinization and iii) discolouration, which are followed by filtration, washing and drying [6, 8, 10].

## 2.3. Chitosan

Chitosan (figure 5) is a copolymer of glucosamine and N-acetyl-glucosamine groups. The ratio between these two groups is called the degree of deacetylation, which is controlled by modifying the time and temperature of the deacetylation process. Commercial chitosan has a degree of deacetylation between 60 and 100 %. [11] This ratio can also explain other physio- and

biological parameters, like density, solubility, mechanical and thermal properties. [6]

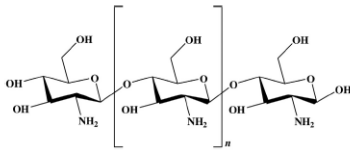


Figure 5 – Chemical structure of chitosan 100 % deacetylated. Adapted from [9].

Thus, chitosan is not highly available in nature, being derived from chitin by a deacetylation process, which frees the amino and hydroxyl groups, chemically active, from chitin. [6]

Its applications are very diverse: it can be used in pharmacy, biomedicine (*e.g.*, drug delivery, tissue engineering, regenerative medicine), agriculture, water treatment, preparation of biodegradable films, coatings and nanocomposites; and it has gained some interests in food and paint applications. [6]

### 3. Materials and Methods

#### 3.1. Raw Materials

The raw material used in this work was insect (black soldier fly, BSF) exuviae, from a biomass by-product without any commercial value that is presently considered agricultural waste.

#### 3.2. From chitin to chitosan

The transformation from biomass to chitosan (Q) was made following a literature procedure [10].

##### 3.2.1. Part I – Extraction of the chitin from the biomass

First, the raw biomass was ground and sifted through a sieve with a mesh of 0.8 mm. Then, a 10 % solution of HCl with a solid-to-solvent ratio of 1:10 (w/v) to the fine biomass was added and stirred for 22 h at room temperature. To the previous mixture, a 10 % solution of NaOH was added with a solid-to-solvent ratio of 1:15 (w/v) and stirred for 24 h at 70 °C.

The mixture was then decolorized with acetone and washed with distilled water. The

final two steps were a filtration and a complete drying of the product (chitin, C) in an oven at 50 °C.

##### 3.2.2. Part II – Producing chitosan from the chitin

Chitin (C) produced in the part I was crushed and sifted with a sieve with a mesh of 0.8 mm. Then a 60 % solution of NaOH was added with a solid-to-solvent ratio of 1:15 (m/v) and stirred for 72 h at room temperature. Chitosan (Q) was washed with distilled water, filtered and dried in an oven at 50 °C.

### 3.3. From chitosan to bioplastic

#### 3.3.1. PG

The process described by Malajovic *et al.*, [12] was followed. Firstly, gelatine and water were heated, at 100 °C, and stirred until the solution became transparent. Then, a solution of glycerol, at room temperature, was added to the previous solution, heated and stirred for 10 minutes. The final product was dried on a foil sheet or on a Petri dish at room temperature in a fume hood for 12 h with extraction, until set. Some changes were made to the above process to change bioplastic mechanical properties: some of the gelatine was replaced by biomass, chitin or chitosan, maintaining the total mass of solids and the same proportion of distilled water and glycerol solution.

When gelatine was partially replaced by chitosan, acetic acid was added to enhance the solubilization process. In this case, the proportion of solid to distilled water and glycerol was kept the same.

The bioplastic was produced by adding glycerol and chitosan solutions to the gelatine one and let it to be stirred and heated for 10 minutes. The final step was drying the formed bioplastic on a foil sheet or on a Petri dish at room temperature in a fume hood, with extraction, for 12 h to 48 h, until set.

Another experimental variation was made by using agar and chitosan and boiling the solution of agar with distilled water for 5 minutes.

## 4. Results and Discussion

Several methods were used to characterize the materials involved in the bioplastics production.

### 4.1. Biomass

#### 4.1.1. SEM/EDS

The biomass (BSF exuviae) presents an irregular surface morphology as detected by SEM (figure 6). This could be because it comes from a larvae cocoon. By SEM/EDS it was possible to know the biomass surficial chemical constitution: it contains carbon, oxygen, calcium, phosphorus, potassium, magnesium, silicon, sulphur, aluminium and chlorine.

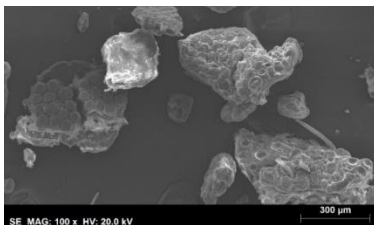


Figure 6 – SEM of the biomass.

#### 4.1.2. FTIR-ATR

BSF biomass FTIR-ATR spectrum (figure 7) exhibits some similarities, in the 1500 and 750  $\text{cm}^{-1}$  range, with the one obtained from a shrimp shell (figure 7). However, chemical bonds may have different interactions in each biomass, since the species are quite different.

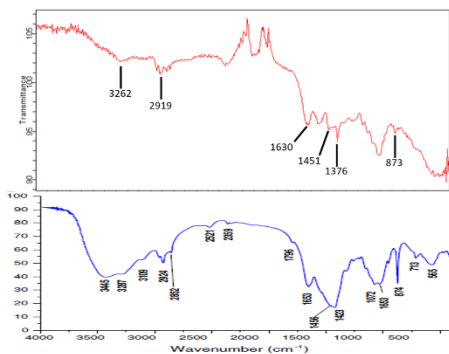


Figure 7 – FTIR-ATR spectrum of the BSF biomass (top) and shrimp shell (bottom, adapted from [13]).

#### 4.1.3. TGA

TGA of the BSF biomass was run from room temperature up to 700  $^{\circ}\text{C}$ , at a heat rate of 20  $^{\circ}\text{C}/\text{min}$ . It was observed a mass loss of 91.5 %,

which is expected because almost all of the sample was from a natural source.

### 4.2. From chitin to chitosan

The first chitosan batch produced, Q1, after drying, led to a film with good mechanical resistance. However, after 2 weeks, Q1 started to curl up and some white spots appeared in spite of being stored protected against sunlight.

Q2 was a Q1 procedure scale-up of 10 times. The final product was easily transformed into a brown and white powder. The next experiments, until Q9, were variations of Q2 procedure, with different BSF biomass amounts and reaction times. The outcome of these samples was very similar – a product that is easily transformed into powder.

On average, in the *part I* of this experiment, each gram of biomass was possible to extract 0.60 g of chitin. On the other hand, in the *part II* of this experiment, the average yield was 34.6 %.

#### 4.2.1. SEM/EDS

SEM revealed that the extracted chitin had in its composition carbon, oxygen, sodium, calcium, chlorine, magnesium, phosphorus, silicon and potassium. These elements were expected since they exist in the biomass and/or were added through the process, like the sodium and chlorine.

The elements present in the chitin samples were also detected in the chitosan product, indicating an incomplete removal of magnesium and calcium from the biomass.

It is important to notice that the device does not detect nitrogen which is expected to be present in all samples.

#### 4.2.2. FTIR-ATR

All FTIR-ATR spectra were run in the 400 and 4000  $\text{cm}^{-1}$  range. When comparing the spectra of chitin (C) samples, some differences in the peak's intensities occur. This also happened to the chitosan's spectra (Q).

The FTIR-ATR spectrum of BSF extracted chitin was also compared to the spectrum of the chitin obtained from shrimp shell (figure 8). The spectrums are very similar, despite the intensity of the peaks.

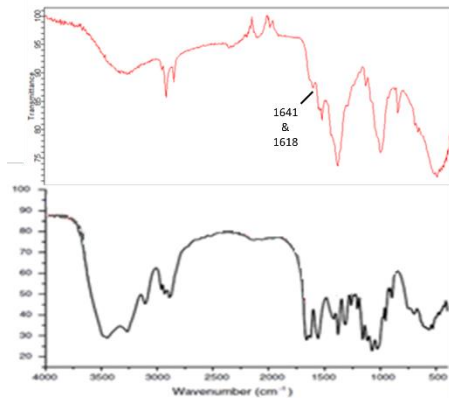


Figure 8 – FTIR-ATR spectrum of the chitin from BSF (top) and chitin from shrimp shell (bottom, adapted from [13]).

The FTIR-ATR spectra of obtained chitosan and commercial chitosan (figure 9) matched perfectly.

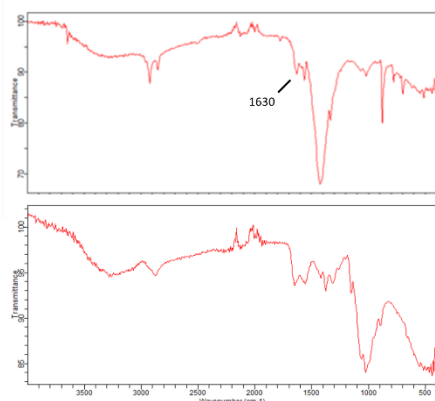


Figure 9 – FTIR-ATR spectrum of the chitosan from BSF (top) and commercial chitosan (bottom).

When the FTIR-ATR spectrum of the chitin and chitosan, produced in this work, is possible to notice that the peak at  $1600\text{ cm}^{-1}$  decrease its intensity, which means that the deacetylation occurred.

#### 4.2.3. Degree of Deacetylation (DD)

The average value of the degree of deacetylation of the chitosan was 71.3 % (FTIR-ATR method). The Sabin's law was also applied with the values obtained in the FTIR-MIR spectrum of Q9.

#### 4.2.4. TGA

The mass loss of C7 and Q5 was 73.5 % and 31.8 %, respectively, indicating that throughout the treatments, biomass to chitosan, the product becomes thermally more stable, as expected.

### 4.3. PG

The difference of PG2 to the samples PG1, PG3 and PG34 was the amount of glycerol used, making PG2 more bendable than the others, that, after drying, become hard films.

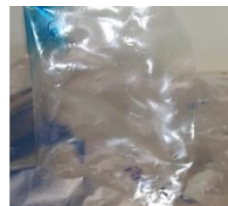


Figure 10 – PG16 after drying for 1 week at room temperature.

The samples PG4, PG5, PG8, PG16, PG18 and PG20 were produced with different amounts of agar. Among them, the best result was achieved in PG16, which resulted in a thin film (figure 10).

PG6, PG7, PG9 to PG15 and PG17 samples were

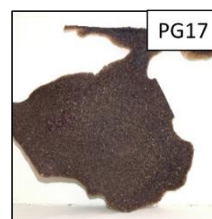


Figure 11 – PG17 after drying.

made with gelatine and biomass in different percentages. The best product was PG17 (figure 11): the biomass loading was 85 % and it forms a uniform film without cracking. Therefore, it was

used as a reference to perform other tests when replacing biomass by chitin or chitosan. All these samples had a scent, which is characteristic of using biomass without any treatment.

PG19, PG21, PG22, PG35 and PG38 were produced with chitosan and gelatine. PG19, PG21 and PG22 didn't have acetic acid incorporated. The products had a crumbly texture and didn't form a film. In PG35 and PG38 (figure 12) acetic acid was incorporated. The sample PG35 was made with 80 % of chitosan and PG38 with 20 % of chitosan. PG35 was a very thin and malleable film, whereas PG38 had a thicker consistency, but it was also malleable.



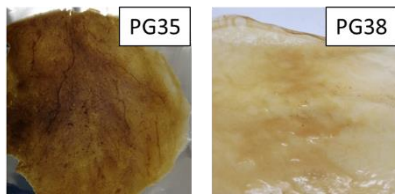


Figure 12 – PG35 and PG38 after drying.

PG23 was made with chitin and gelatine. This sample had to dry for 4 days in the fume hood, with extraction. The result was a film that on the top is matt and on the bottom is shiny.

PG37 was an attempt to mix biomass with agar. The result was a film with multiple cracks.

PG24 and PG36 were made with chitin and agar. The result was a film with multiple cracks.

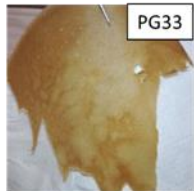


Figure 13 – PG33 after drying.

To test the chitosan with agar PG25, PG26, PG27, PG30, PG33, PG33.2 and PG33.3 were produced. PG26 had the best outcome, a film with some resistance and a light colour. Then, it was made scale-up of three times of this sample (figure 13): PG33.2 and PG33.3. The result was a 23 cm diameter film. All samples with chitosan and chitin didn't present an odour, since chitosan is an already a transformed biopolymer.

#### 4.3.1. SEM/EDS

PG17 contains oxygen, calcium, carbon, potassium, silicon, magnesium, phosphorus and sulphur. PG23 has carbon, chloride, sodium, oxygen, calcium, sodium, potassium, phosphorus, magnesium, silicon, sulphur and aluminium. In PG33, there are carbon, oxygen, sodium, calcium, chloride, silicon and magnesium. These elements were expected for all samples, since they all exist in the polysaccharide.

Figure 14 shows that the surface of PG3 film has some droplets, which can be expected due to the water used in its production process.

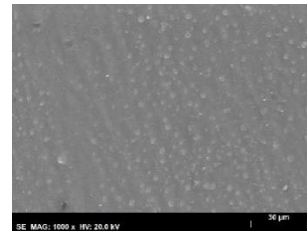


Figure 14 – SEM of PG3.

#### 4.3.2. FTIR-ATR

The best samples obtained were PG33 (figure 15) and PG38 (figure 16). The first sample spectrum – made with chitosan and agar (figure 15) – was compared to agar spectrum revealing that in the 1600 – 4000  $\text{cm}^{-1}$  range they are very similar. On the other hand, in the range of 400 – 1500  $\text{cm}^{-1}$ , PG33 spectrum has some similarities to the chitosan one. The other peaks that do not correspond to either spectra can be justified by reactions that occur between the reactants.

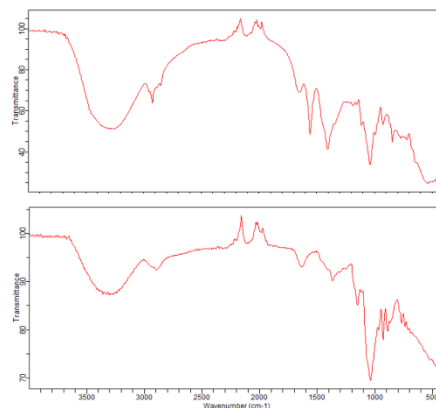


Figure 15 – FTIR-ATR spectra of PG33 (top) and agar (bottom).

PG38 – made with chitosan and gelatine (figure 16) – when compared to the gelatine and chitosan spectrum have similar differences as PG33, which can have the same justification.

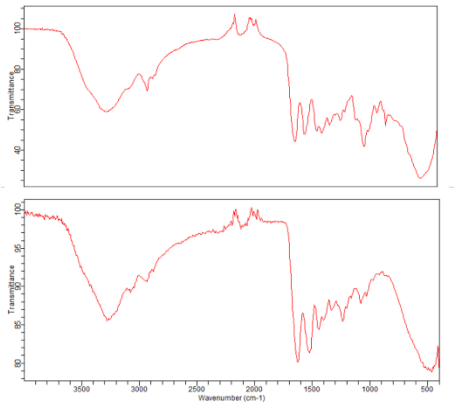


Figure 16 – FTIR-ATR spectra of PG38 (top) and gelatine (bottom).

#### 4.3.3. TGA

Table 1 shows that, regardless of its polysaccharide, the PG's samples degrade thermally. This can mean that the addition of these compounds changed the structure of the film.

Table 1 – Mass loss percentage of some samples of PG.

Sample	PG3	PG6	PG7	PG9	PG10	PG11
% loss	93.5	82.9	92.3	82.1	80.6	86.2
Sample	PG16	PG23	PG26.f	PG28	PG29	PG33
% loss	96.2	78.3	80.0	51.6	55.0	61.8

#### 4.3.4. Mechanical tests

The strain of some samples was determined. Samples that have gelatine (G) and biomass (B) were compared (figure 17). There isn't a linear behaviour relating the strain and the percentage of gelatine present in the sample. Moreover, the sample with only gelatine has a higher strain value.

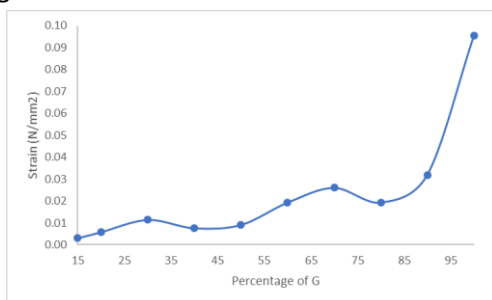


Figure 17 – Strain vs. % of gelatine in the sample with gelatine and biomass.

For samples made with gelatine (G) and biomass, chitin (C) or chitosan (Q) it was found that the higher value corresponds to the sample with only gelatine (figure 18). Then,

samples PG17, PG23 and PG35 have the same quantity of gelatine substitute, but they don't exhibit similar strain results. Nevertheless, when comparing the first two samples PG38 they have similar results, that is when the samples have both gelatine and chitosan. It is necessary to increase the amount of gelatine to obtain a higher value of strain.

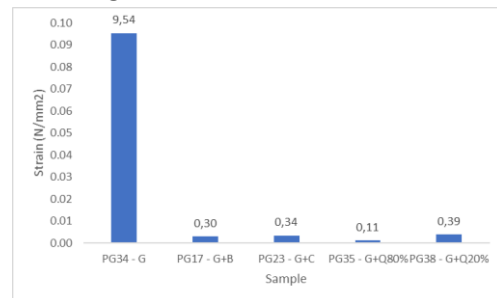


Figure 18 – Strain vs. sample with polysaccharide and gelatine.

The final evaluation performed with the strain was with the samples with agar (figure 19). In this case, if there's a polysaccharide added to the mixture the strain decreases. This can happen because the chemical bonds can change with the addition of some compounds.

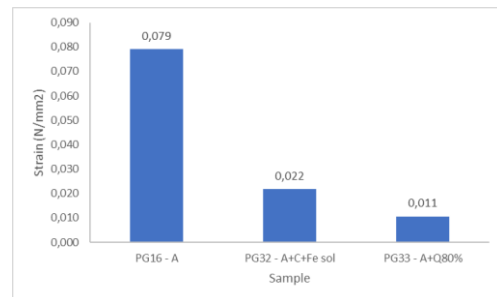


Figure 19 – Strain vs. sample with polysaccharide and agar.

#### 4.3.5. Stability tests

The stability test was performed using PG33 under different gaseous atmospheres, nitrogen, air and carbon dioxide. The samples stayed in the flasks for 114 days and they didn't lose any weight or colour. When PG33 was left under direct sunlight for 106 days, it shrunk, its colour turned into white and, because it was not covered, it had some black particles, probably dust (figure 20).



Figure 20 – PG33 before (left) and after (right) left under direct sunlight.

#### 4.3.6. Formation of Moulds

PG33 was also tested to see if it was possible to obtain moulds with it. The procedure was not successful, as the solution had shrunk or didn't dry completely. However, it printed the mould and held some shape (figure 21 and 22).



Figure 21 – Cupcake mould.



Figure 22 – Beaker mould.

## 5. Conclusion and Future Work

The main goal of this work was to develop a bioplastic from an agri-food waste. This goal was achieved by creating a film, however, tests are required to conclude if this bioplastic is biodegradable or not.

SEM images of extracted chitin and produced chitosan revealed an irregular morphology. Moreover, as expected, all components of the biomass were detected at the surface of extracted chitin and produced chitosan. However, it was not possible to quantify such components.

The average degree of deacetylation of the produced chitosan samples was 71.3 %. This value confirms the solubility of the chitosan in dilute acids.

With TGA analysis it is possible to verify that, though the process of transforming the biomass into chitosan, each compound (biomass, chitin and chitosan) becomes more

thermally stable. This can entail some benefits, such as, the shelf time of these products can increase. However, it can have some disadvantages, like not being biodegradable or eco-friendly, like fossil fuel-based plastics. Without performing the degradation tests, it cannot be concluded.

The method used to produce the bioplastic – PG – had successful outcomes. TGA analysis revealed that most of the samples are very degradable, which can be a positive aspect and can place this film in the biodegradable category. In the SEM images it was possible to see that the PG is indeed a cohesive film.

It was possible to produce a strong PG film and other more flexible and thinner, with chitosan and gelatine. Nevertheless, the first sample had 20 % of the total mass of solids and the second had 80 %. So, it is convenient to try and find a proportion that can offer both options.

Either for samples with gelatine or agar, when there is no polysaccharide added, the strain is higher. Overall, the samples that have agar have a lower strain and there is no direct correlation between the percentage of gelatine in the sample and its strain.

It was possible to do some moulds with these samples, mainly with PG33. However, they did not come in one piece or they didn't dry enough to be in one piece. Therefore, it is necessary to create a way to make these moulds more efficiently.

For future work, it is important to test different reactants in order to improve the process of transforming biomass into chitosan. This means that the process could be more efficient, be more environmentally friendly or have fewer costs associated.

Another suggestion for future work is to improve the glycerol, gelatine, agar and acetic acid (when applied) ratios to achieve flexibility and strength for the film, as well as, test its biodegradability. Finally, it would be interesting to explore other applications for the chitosan produced from the BSF exuviae.



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