doi:10.20944/preprints202212.0344.v1

Article

Plasticized Mechanical Recycled PLA Films Reinforced with Microbial Cellulose Particles Obtained from Kombucha Fermented in Yerba Mate Waste

Ángel Agüero [1,2], Esther Corral Perianes[2], Sara Soledad Abarca de las Muelas[2], Diego Lascano[1], M. M. de la Fuente García-Soto[2,3], Mercedes A. Peltzer [3,5], Rafael Balart[1] and Marina P. Arrieta [2,6,*]

[1] Instituto de Tecnología de Materiales (ITM), Universidad Politécnica de Valencia (UPV), Plaza Ferrándiz y Carbonell 1, Alcoy 03801, Spain; anagrod@epsa.upv.es (A.A.); dielas@epsa.upv.es (D.L.); rbalart@mcm.upv.es (R.B.)

[2] Departamento de Ingeniería Química Industrial y del Medio Ambiente, Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), Calle José Gutiérrez Abascal 2, 28006 Madrid, Spain.; angel.agrodriguez@externos.upm.es (A.A.); esther.corralp@alumnos.upm.es (E.C.P.); ss.abarca@alumnos.upm.es (S.S.A.M.); mariadelmar.delafuente@upm.es (M.M.F.G-S); m.arrieta@upm.es (M.P.A.)

[3] Grupo de Investigación: Tecnologías Ambientales y Recursos Industriales (TARIndustrial), 20006 Madrid, Spain.; mariadelmar.delafuente@upm.es (M.M.F.G-S)

[4] Laboratory of Obtention, Modification, Characterization, and Evaluation of Materials (LOMCEM), Department of Science and Technology, University of Quilmes, Bernal B1876BXD, Argentina. mercedes.peltzer@unq.edu.ar (M.A.P.)

[5] National Scientific and Technical Research Council (CONICET), Buenos Aires C1425FQB, Argentina. mercedes.peltzer@unq.edu.ar

[6] Grupo de Investigación: Polímeros, Caracterización y Aplicaciones (POLCA), 28006 Madrid, Spain.; m.arrieta@upm.es (M.P.A.)

* Correspondence: m.arrieta@upm.es (M.P.A.); Tel.: +34-910-677-301

Abstract: Yerba mate waste (YMW) were used to produce kombucha beverage, and the obtained microbial cellulose produced as by product (KMW) was used to reinforce mechanically recycled poly(lactic acid) (r-PLA) matrix. Microbial cellulosic particles were also produced in pristine yerba mate for comparison (KMN). To simulate the revalorization of industrial PLA products rejected during the production line, PLA was subjected to three extrusion cycles and the resultant pellets (r3-PLA) were then plasticized with 15 wt.% of acetyl tributyl citrate ester (ATBC) to obtain optically transparent and flexible films by solvent casting method. The plasticized r3-PLA-ATBC matrix was then loaded with KMW and KMN in 1 and 3 wt.%. The use of plasticizer allowed a good dispersion of microbial cellulose particles into the r3-PLA matrix, allowing to obtain flexible and transparent films which showed good structural and mechanical performance. Additionally, the obtained films showed antioxidant properties, as it was proven by release analyses conducted in direct contact with a fatty food simulant. The results suggest the potential interest of these recycled and biobased materials that are obtained from the revalorization of food waste for their industrial application in food packaging or agricultural films.

Keywords: PLA; cellulose; yerba mate; kombucha; food packaging

1. Introduction

Biobased and biodegradable polymers have gained attention for food packaging applications in order to reduce the consumption of non-renewable resources and prevent the accumulation of plastic waste in the environment. Among other biopolymers, poly(lactic acid) has positioned in the market as the most used biobased and biodegradable plastic due to its many advantages such as its environmentally benign characteristics, availability in the market at a competitive cost, ease of processing by means of the current existing processing technologies for petrol-based thermoplastics (i.e.: extrusion, injection molding, etc.), high transparency, and its inherent biodegradability [1, 2]. However, PLA also presents some disadvantages for film production which hinder its industrial exploitation in the food packaging or agricultural sectors such as its sensitivity to thermal degradation [2], its poor barrier performance [3], its inherently brittle nature [4], and its degradability in the environment requires specific conditions (compost medium at 58 °C, a pH around 7.5, relative humidity of 60%, a C/N relationship between 20:1 and 40:1, and proper aeration) to take place in shorts periods [5]. Moreover, the model of the linear economy generates high levels of plastic waste and creates a dependence between economic development and the entry of new virgin plastics into the system [6]. Therefore, the use of recycled PLA for film for food packaging or agricultural applications is gaining interest [7, 8]. Cosate de Andrade et al. [8] have compared the chemical recycling and mechanical recycling of PLA and have concluded that mechanical recycling generates less impact than chemical one, due to the fact that the mechanically recycled polymers are produced using lower energy and inputs than other destinations. However, bioplastic consumption is currently still low and they can be considered contaminants in plastics recycling streams, due to the fact that they can affect the mechanical performance of other well implemented mechanical recycling process of other plastics such as polyethylene terephthalate (PET), polypropylene (PP) and polystyrene (PS) [9-11]. Moreover, although the European Commission in its strategy to introduce recycled plastics in a circular economy promotes the increase of recycled plastic in food packaging as an essential prerequisite, the current legislation does not allow the direct use of recycled plastics coming from recycled streams for food contact materials since those recycling processes originate from waste, and the legislation establishes strict requirements concerning food safety (the transfer of substances that may affect human health, or quality of the food, and microbiological safety) [12]. In this context, during the industrial production of plastics products several parts are produced with some defects and are rejected from the production line being then discarded. Those rejected parts can be reprocessed and used to produce recycled pellets that do not come from waste streams and are of well-known origin. In previous work, PLA was reprocessed up to six times and it was observed that the main losses took place when PLA was subjected to more than four reprocessing cycles, while low degradation was found between 1 and 3 reprocessing cycles [13]. However, due to the PLA high sensitivity to hydrolysis of its ester groups at the industrial processing conditions such as melt-extrusion, the obtained recycled PLA-based products shows a decrease in the polymer chain length and, thus, have lower performance with respect to PLA-based products produced with virgin PLA [14]. This is why the use of reinforcing fillers with antioxidant activity as additives have gained interest to protect the polymeric matrix from thermal degradation and increase the mechanical resistance of mechanically recycled PLA [7].

Another industrial sector that generates a high amount of waste that can be introduced in the food packaging sector for the preparation of high-tech composites and/or nanocomposites is the food industry [15]. Kombucha beverage is a popular probiotic beverage typically produced by fermenting sugared tea with a symbiotic community of bacteria and yeast (SCOBY) that involves cooperative and competitive interactions [16]. While yeasts produce invertase that releases monosaccharides to media accessible to any microbe as a carbon source, bacteria rapidly metabolise released sugars and produce organic acids that produce the acidifications the media [16]. In the meantime, the reduction of monosaccharides increases the frequency of the invertase-producing yeast and the ethanol produced by yeast stimulates the bacterial cellulose-synthase mechanism to produce a cellulose film at the surface that is a physical barrier protect from external competitors [16]. The cellulosic film is a byproduct in the kombucha tea industry, but it is very interesting for the plastic industry. Kombucha tea has been fermented in several sugared infusions (i.e.: black tea, green tea, yerba mate etc.) [16-18]. The antioxidant activity of microbial cellulose obtained from the kombucha fermentation is directly related with the high amount of bioactive compounds in the infusion used for its fermentation such as phenolics, tannins, catechins, flavonoids, etc., which are decomposed into their simpler forms

doi:10.20944/preprints202212.0344.v1

during the kombucha fermentation process [17]. In fact, tt has been observed that the cellulose obtained from kombucha fermented in sugared infusion of yerba mate possess high antioxidant activity [16]. Yerba mate (*Ilex paraguariensis*, Saint Hilaire) is a tree from the subtropical region of South America, that grows in a limited zone within Argentina, Brazil and Paraguay, where it has an important commercial activity due to the high consumption of yerba mate dried leaves in the form of infusion which is known as "mate"[15, 19]. Its high consumption leads to a high amount of yerba mate wasted without any kind of revalorization [15]. For instance, in 2020 the consumption of yerba mate in Argentina was over 310 million kg which [20]. Thus, in this work kombucha SCOBY was fermented in yerba mate waste.

Among others plasticizers, citrate esters such as acetyl(tributyl citrate) (ATBC) have proven to be very effective PLA plasticizers and are accepted for food contact applications [21]. The miscibility between PLA and ATBC has been associated with the similarity in their solubility parameters (δ) being that of PLA between 19.5 $MPa^{1/2}$ and 20.5 $MPa^{1/2}$ [2], while that of ATBC is 20.2 $MPa^{1/2}$ [22]. Likewise, to produce polymers by solvent casting method the selection of an effective solvent is also on the basis of a similar solubility parameter to that of the polymer. In this sense, chloroform (δ = 19 $MPa^{1/2}$) is widely used to dissolve PLA [23].

In this work, virgin PLA was subjected to 3 reprocessing extrusion cycles (r3-PLA) since in a previous work it was observed that between 1 and 3 reprocessing cycles low PLA degradation occurs [13]. The decrease in the polymer chain length due to the 3 reprocessing cycles was investigated by the measurement of the viscosity molecular weight. On the other side, yerba mate waste was used to obtain the sugared infusion to produce kombucha beverage from Kombucha SCOBY, while the cellulosic by-product formed during its production was used to produce cellulosic particles with antioxidant activity (KMW). Another Kombucha SCOBY was fermented in a sugared infusion of new yerba mate and the cellulosic particles obtained were studied for comparison (KMN). Both particles, kombucha mate waste (KMW) and kombucha mate new (KMN), were used to reinforce plasticized mechanically recycled r3-PLA with 15 wt.% of ATBC. Two reinforcing amounts were used 3 wt.% and 5 wt.% and the obtained films were characterized in terms of transparency, barrier performance against water and UV light, thermal stability, crystallization behavior, surface wettability as well as the mechanical performance in order to get information regarding the possibility of using these films as food contact materials such as food packaging or in the agro-industrial field.

2. Materials and Methods

2.1. Materials

PLA commercial-grade IngeoTM 2003D with a density of 1.24 $g\cdot cm^{-3}$ and a melt flow index (MFI) of 6 g/10 min (measured at 210 °C and with a load of 2.16 kg) was supplied by Natureworks (Minnetonka, MN, USA). Acetyl tributyl citrate (ATBC) (98% purity, Mw = 402 g mol^{-1}, and Tm = −80 °C), chloroform ($CHCl_3$, δ = 19 $MPa^{1/2}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) 95% free radical were supplied by Sigma Aldrich (Madrid, Spain). The pristine yerba mate (Taragüi, Argentina) was used as it is and named YMN, while the yerba mate waste was obtained from the residue of mate infusion after our consumption and named YMW.

2.2. Processing of kombucha to obtain cellulosic particles from yerba mate waste

The native culture of Kombucha was provided by Teresa Carles Manufacturing S. L. (Barcelona, Spain) used as the starter culture and used as inoculum of a new batch of kombucha fermentation in an infusion of yerba mate (5 g/L) and sucrose (100 g/l). KMW was obtained from the fermentation of one kombucha SCOBY from that batch in a 2.5 L sugared infusion prepared either with 15 g of yerba mate (YMN) and/or yerba mate waste (YMW), 300 g of sucrose, and 500 ml of stock culture which was maintained at static conditions at 22 ± 2 °C covered with a textile cloth for 30 days. A new floating disc was produced and it was recovered washed with distilled water, filter off and further sterilized at 121°C and 101 kPa for 15 min in a steam autoclave. The disc was then homogenized by ultraturax at 30,000 rpm for two minutes (4 cycles of 30s) and dried at 60 °C for 24h. The dry matter, determined by drying at 105 °C until constant weight, showed a yield of ca. 1.3 ± 0,1%, in good accordance with previous reported works [16]. Then the cellulosic paper obtained was grinded to obtain a powder and further sieved (500 µm). In **Figure 1** is schematically represented the wall process to obtain either KMN or KMW from the SCOBY fermented in YM or YMW to the powder able to be processed by melt extrusion.

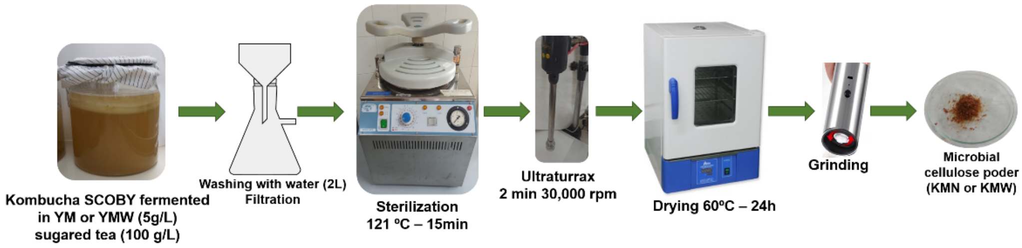

Figure 1. Schematic representation of the microbial cellulose (KMN and/or KMW) production from kombucha fermented in YM or YMW.

2.2. Processing and Reprocessing of PLA

To obtain reprocessed PLA (r3-PLA), PLA pellets was previously dried overnight to remove the residual moisture at 60 °C for 4 h in an air-circulating oven. The PLA pellets were processed 3 times in a twin-screw co-rotating extruder with a screw diameter of 30 mm, supplied by Construcciones Mecanicas Dupra, S.L. (Alicante, Spain) at screw speed at around 22 rpm and using a temperature profile of: 180 °C (feeding hopper), 185 °C, 190 °C, and 195 °C (extrusion die) on the basis of previous work [13]. After the extrusion process, the strands were cooled in air and then pelletized using an air-knife unit and were then subsequently subjected to an additional processing cycle under the same conditions up to three times.

The capillary viscosity of virgin PLA and r3-PLA pellets was measured with a Ubbelohde viscometer (type 1C). Both pellets were diluted in $CHCl_3$ and the measurements were conducted at 25 °C using a water bath and a home-made 3D printed viscosimeter support. At least four concentrations were used. The intrinsic viscosity [η] of PLA and r3-PLA was determined to estimate the viscosity molecular weight by means the Mark-Houwink relation (Equation 1).

$$[\eta] = K \times M_v^a \tag{1}$$

were *K* and *a* for PLA are 1.53×10^{-2} and 0.759, respectively [24].

2.3. Films preparation

KMN and KMW loaded r3-PLA-ATB-based materials were processed into thin films by solvent casting method. For this purpose, 0.6 g of reprocessed PLA pellets (r3-PLA) were dissolved in 45 mL of $CHCl_3$ under continuous stirring at 1000 rpm at room temperature. ATBC was then added in 15 wt.% with respect to the polymeric matrix, on the basis of previous works [15, 22, 25], and named as r3-PLA-ATBC. For the development of composites, the plasticized PLA films (r3PLA-ATBC) were then loaded either with kombucha mate waste (KMW) or kombucha mate new (KMN) in 1 wt.% and 3 wt.% with respect to the r3-PLA-ATBC polymeric blend and all films were prepared by solvent casting method. Each suspension was cast onto a 50 mm-diameter glass mould and then $CHCl_3$ was allowed to evaporate at 40 °C for 48h in an oven. The obtained films are summarized in Table 1 and they were dried under a vacuum to complete the drying process ensuring the complete elimination of the solvent for about 10 h at 40 °C previous to being characterized.

doi:10.20944/preprints202212.0344.v1

Table 1. Films formulations based on plasticized 3r-PLA-ATBC.

Sample	r3-PLA (wt.%)	ATBC	KMN (wt.%)	KMW (wt.%)
r3-PLA	100	-	-	-
r3-PLA-ATBC	85	15	-	-
r3-PLA-ATBC-KMN1	84.15	14.85	1	-
r3-PLA-ATBC-KMN3	82.45	14.55	3	-
r3-PLA-ATBC-KMW1	84.15	14.85	-	1
r3-PLA-ATBC-KMW3	82.45	14.55	-	3

2.3. Characterization of the films

2.3.1. UV-visible measurements

The transmittance of the obtained films was measured in the 800-250 nm region using a UV-Visible spectrophotometer Varian Cary 1E UV-Vis (Varian, Palo Alto, CA, USA) at a scanning speed of 400 nm/min. The overall transmittance in the visible region was calculated following the ISO 13468 standard.

2.3.2. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analyses were conducted in a Mettler-Toledo model 821 DSC (Schwerzenbach, Switzerland). The DSC thermal cycles were carried out, under a nitrogen atmosphere. The first heating DSC scan was conducted from 30 °C to 200 °C at a rate of 10 °C/min with the main objective of eliminating the thermal history. Then, the samples were cooled down to -50 °C at a rate of 10 °C/min. Finally, the second heating DSC scan was carried out from -50 °C to 300 °C at a rate of 10 °C/min. The degree of crystallinity (χ_c), obtained from the DSC thermograms, was calculated through Equation 2.

$$\chi_c = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \cdot \frac{1}{W_{PLA}} 100 \qquad (2)$$

where ΔH_m is the melting enthalpy, ΔH_{cc} is the cold crystallization enthalpy, ΔH_m^0 is the melting heat associated with pure crystalline PLA (93 J g^{-1}) [26] and W_{PLA} is the weight fraction of PLA in the blend formulation.

2.3.4. Thermogravimetric analysis

Dynamic thermogravimetric analyses were conducted in a TA Instruments TGA2050 thermobalance. For each measurement, around 10 mg of films were put in a platinum crucible and heated from 30 to 800 °C, at 10 °C/min under nitrogen atmosphere.

2.3.5. Tensile test measurements

The mechanical properties were measured by means tensile test measurements using a Shimadzu AGS-X 100N universal tensile testing machine (Shimadzu Corpo-194 ration, Kyoto, Japan) equipped with a 100N load cell, using an initial length of 30 mm at a cross-head speed of 10 mm min^{-1}. Dog-bone samples were prepared by a JBA electrohydraulic cutter (Instruments J. Bot SA) for tensile specimens 1BB according to ISO 527-2. The Young modulus (E) and tensile strength (TS) and average percentage elongation at break (ε%) were calculated from the obtained stress-strain curves and the media of at least five specimens were reported.

2.3.6. Static Contact Angle Measurements

Surface wettability of films was studied through static water contact angle (WCA) measurements by using a standard goniometer (EasyDrop-FM140, KRÜSS GmbH, Hamburg, Germany) equipped with a camera and Drop Shape Analysis SW21; DSA1 software. Drops of ~15 μL distiller water were put onto the films' surfaces with the aid of a syringe and around ten contact angle measurements were taken for each sample in random positions of the films.

2.3.7. Water Vapour Transmission Rate

The water vapor transmission rate (WVTR) measurements of the films were determined by gravimetry using silica gel as a desiccant agent. Films were placed in permeability cups, with an exposed area (A) of 10 cm^2, filled with 2 g of previously dried silica gel, and further placed in a desiccator at 23 ± 1°C with a saturated KNO_3 solution obtaining a relative humidity of 85±4 %. The cups were weighed every hour for 7 h and then after 24h. The mass increase of the cups was plotted against time, with slope n. WVTR (g/day cm^2) was determined through Equation 3:

$$WVTR = \frac{n}{A} \tag{3}$$

Because the water vapour transmission is dependent on the film thickness the WVTR values were normalized to 100 μm [27].

2.3.7. Specific migration test and antioxidant activity

Double-sided total immersion migration tests were performed by total immersion of films in a glass vial containing a fatty food simulant (Simulant D1 = ethanol 50% v/v), at 40 °C for 10 days (area-to-volume ratio = 6 dm^2/L) [28]. After 10 days films were removed and the food simulant was used to determine the antioxidant ability of films which was measured by the determination of the radical scavenging activity (RSA) through the DPPH-method by the determination of the reduction of the absorbance at 517 nm by means of a UV-Vis Varian Cary spectrophotometer. The radical scavenging activity (RSA) was determined using the Equation 4.

$$RSA\ (\%) = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100\% \tag{4}$$

where $A_{control}$ is the absorbance of 2,2-difenil-1-picrylhydrazyl (DPPH) in ethanolic solution and A_{sample} the absorbance of DPPH after 15 min in contact with each food simulant sample.

3. Results

3.1. Reprocessed PLA characterization

The materials developed here were prepared with mechanically recycled PLA which was processed 3 times by melt extrusion using a temperature profile from feeding to hopper of: 180 °C, 185 °C, 190 °C, and 195 °C based on previous work [13], to simulate the revalorization of industrial waste produced during the production line in which some parts are rejected. The viscosity molecular weight (M_v) of reprocessed PLA (3r-PLA) and PLA pellets was determined to get insights on the degradation of the polymeric matrix as a consequence of reprocessing procedure. The obtained results of the estimated M_v of PLA and r3-PLA were 181,770 ± 3,370 g/mol and 115,410 ± 5,080 g/mol, respectively (a reduction of around 36%). A reduction in the intrinsic viscosity ($[\eta]$) has been already reported in PLA samples subjected to a simulated mechanical recycling process in which one melt-extrusion reprocessing cycle was applied, showing a reduction of around 14% with respect to samples prepared with virgin PLA [29]. In the present work, a reduction in the intrinsic viscosity ($[\eta]$) due to the 3 reprocessing melt-extrusion cycles was around 30 %, leading to the above-mentioned reduction in the M_v. This reduction in the M_v is due to chain scission produced by thermal degradation during each thermal processing cycle as a consequence of a hydrolysis process which is accentuated by the heating [13].

3.2. UV-visible measurements

The transmittance of the obtained films was measured by means of a UV-Visible spectrophotometer and the absorption spectra of films are shown in **Figure 2**. Neat PLA film was also analyzed for comparison. From the spectra, it could be seen that although all formulations based on recycled PLA (r3-PLA) resulted in less transparent materials than PLA, they were mostly transparent in the visible region of the spectra (400-700 nm) allowing seeing through the films, which is one of the most important requirements for food packaging due to the consumers' acceptance [30] and it is also very important in films intended for agricultural applications were films not only should protect crops but also they should permit the photosynthesis process [31]. Among reprocessed films, r3-PLA film was the most transparent film showing the highest transmission along the visible region of the spectra (400–700 nm). The incorporation of ATBC slightly affected the transparency of r3-PLA as was already observed with the addition of ATBC to virgin PLA matrix [22]. The transparency was slightly reduced with the incorporation of KMN and/or KMW. Absorption measurements were conducted in the range of 540-560 nm (see zoom image in **Figure 2**) of the visible region of the spectra and it can be seen that the materials resulted in highly transparent (between 81% and 87% of transmittance).

When comparing r3-PLA with neat PLA film, it can be observed a slightly UV-light absorption in the 260 to 290 nm region in 3r-PLA sample, which has been already observed in recycled PLA [7, 14]. It has been reported that recycled PLA produces a reduction of the UV light transmission in the 260 to 290 nm region of the spectra, ascribed to the formation of -COOH chain end groups in PLA as a consequence of the chain scission (carbonyl carbon-oxygen bond cleavage) during thermal processing [14]. Nevertheless, it should be highlighted that the UV light transmission reduction is less marked than in postconsumer mechanically recycled PLA bottles studied by Chariyachotilert et al. They observed higher UV light transmission reduction and it can be related not only with the thermal degradation during reprocessing but also with the degradation of PLA product during service as well as during the cleaning conditions typically used for cleaning PET (85°C, 1 wt.% NaOH and 0.3 wt.% Triton® X-100 surfactant for 15 min) [14]. In this sense, in a previous work, Agüero et al. deeper studied the mechanically recyclability of injected moulded PLA parts performing between 1 and 6 reprocessing melt-extrusion cycles and showed that low degradation takes place between 1 and 3 reprocessing cycles of [13]. Thus, it means that less degradation had taken place after 3 reprocessed melt extrusion cycles than in post-consumed, washed and, further reprocessed PLA, highlighting the viability of mechanically recyclability of rejected PLA parts from the production line.

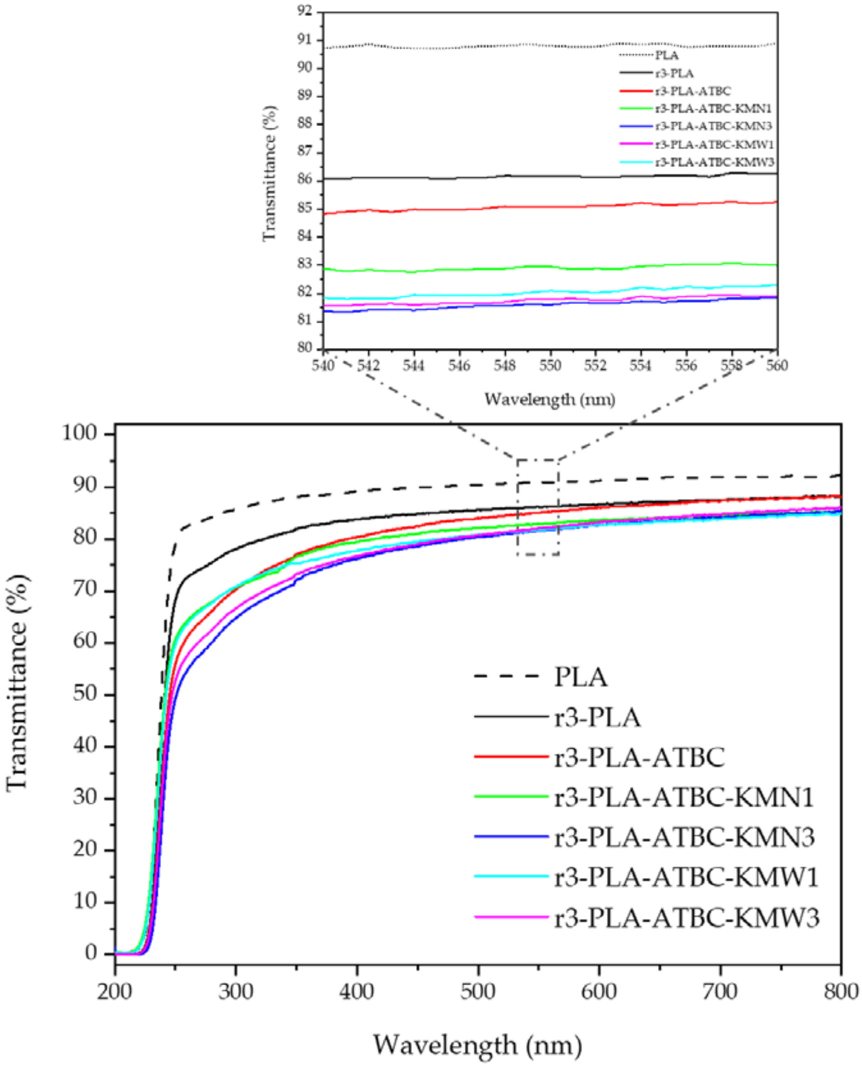

Figure 2. UV–vis spectra of films 200-800 nm and zoom image 540-560 nm.

3.3. Scanning Electron Microscopy

FESEM investigations were conducted to study the microstructure of the films and the micrographs of the cross-fractured surface are shown in Figure 3. The r3-PLA film (Figure 3-a) showed the typical regular and smooth fracture of PLA films based on semicrystalline virgin PLA [9, 32]. An increased ductile fracture was observed in r3-PLA-ATBC film (Figure 3-b) with a more plastic behavior and no apparent phase separation, demonstrating the plasticizing effect of ATBC into the reprocessed PLA matrix. The ternary composites for both, the formulations with 1 wt.% (Figure 3-c and Figure 3-e) and 3 wt.% (Figure 3-d and Figure 3-f), showed a uniform dispersion of both, KMN and KMW, into the r3-PLA matrix. In the case of the higher amount of reinforcing amount used here of 3 wt.% (Figure 3-d and Figure 3-f), it seems that there is an increase of surface roughness but with a very similar surface pattern than that of those reinforced with less amount of kombucha particles of 1 wt.% suggesting that cellulosic particles are well distributed into the reprocessed PLA matrix. It has been observed that plasticizers such as ATBC improve the dispersion of cellulosic particles into the PLA matrix [25].

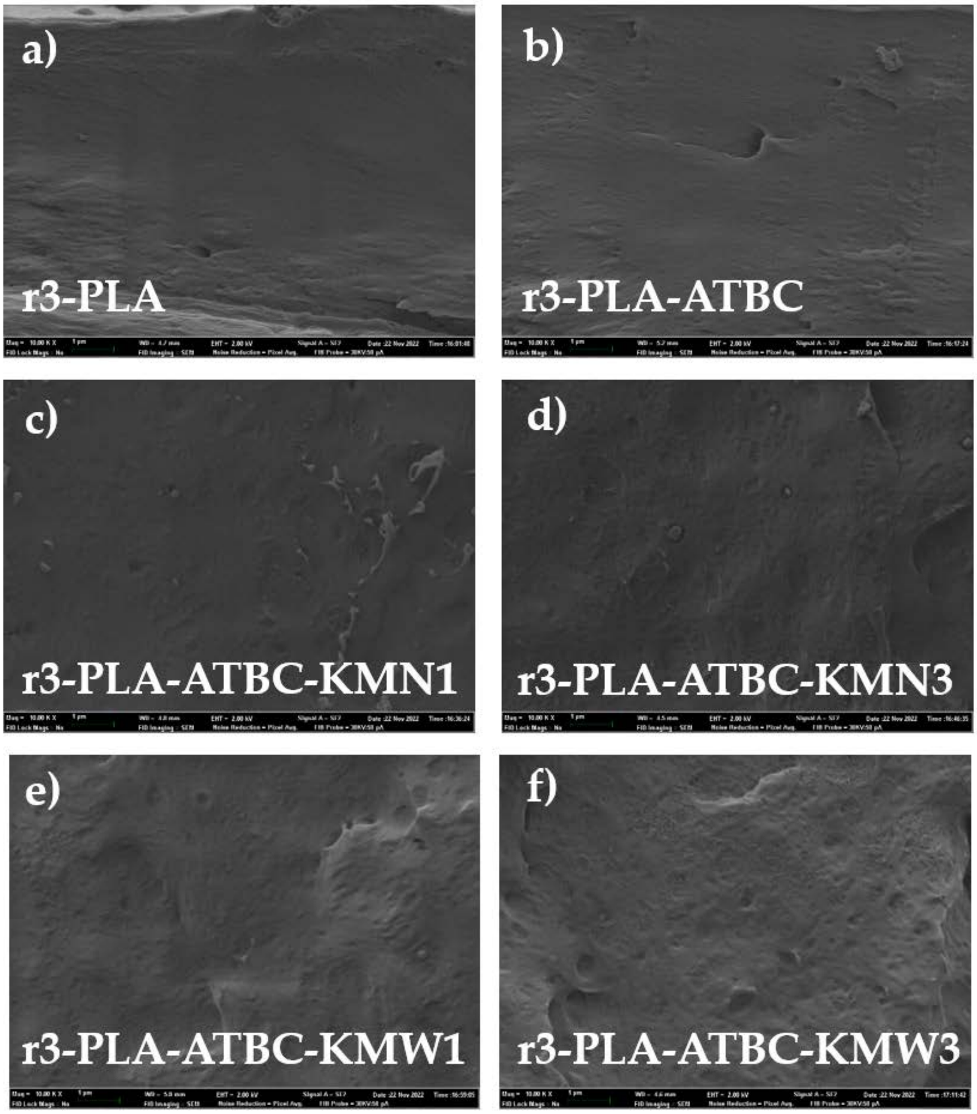

Figure 3. FE-SEM observations at 10,000 x of 3r-PLA-ATBC based films: a) r3-PLA, b) r3-PLA-ATBC, c) r3-PLA-ATBC-KMN1, d) r3-PLA-ATBC-KMW1, e) 3r-PLA-ATBC-KMN3, f) r3-PLA-ATBC-KMW3.

3.4. *Differential Scanning Calorimetry*

DCS analysis was conducted and used to investigate the glass transition (T_g), cold crystallization (T_{cc}), melting temperatures (T_m) and crystallinity (χ_c) or plasticized 3r-PLA-ATBC films, and the obtained DSC curves are shown in Figure 4 while the obtained results are summarized in Table 2. r3-PLA film showed the T_g at lower values than PLA samples processed 3 times by melt-extrusion and further processed by injection moulding (T_g = 64 °C [13]), due to the presence of a residual solvent, as it was demonstrated by Yang et all. who compared PLA-based composites processed by extrusion with those processed by solvent casting method and concluded that limited variations on DSC parameters were observed for samples processed with the two different processing techniques (melt-extrusion and solvent casting method) [33]. In r3-PLA film appeared a cold crystallization peak which was not present in the virgin PLA pellet [13], and it has been related to the fact that the shorter PLA chains formed during the reprocessing cycles such as oligomers had higher mobility and promotes the crystallization of PLA [13, 29]. In fact, it has been observed that PLA plasticized with oligomeric lactic acid (OLA) showed a reduction in the cold crystallization temperature which is further reduced with increasing the OLA content [34, 35]. Similarly, the incorporation of ATBC plasticizer produced a decrease in the T_g, T_{cc} as well as in the T_m, ascribed to the ability of ATBC plasticizer to increase the free volume between the polymer chains and accordingly their mobility enhancing its slow crystallization rate [22, 36]. On the other side, the combination of the ATBC plasticizer and the microbial cellulose particles onto plasticized 3r-PLA matrix produced higher T_g and T_{cc} values and higher crystallinity degrees suggesting that the segmental motion of PLA matrix may have been affected by the presence of KMN and KMW [33]. Moreover, the synergic effect on the crystallization of PLA as a consequence of a potential nucleating agent in a presence of a citrate ester plasticizers has been already reported [25, 37]. The DSC thermograms show a double melting behavior, which has been already observed in mechanically recycled PLA [38, 39] and PLA plasticized with OLA [34]. This behavior in PLA-based materials is ascribed to the presence of different crystalline structures with different perfection and thermodynamic stability. From the melt PLA crystallizes at temperatures higher than 120 °C in an ordered form (α form) [40]. In the present work all samples, crystallized at temperatures below 120 °C that is related with the ability of shorter polymer chains (oligomers) produced as a consequence of the PLA degradation during reprocessing steps that are able to promote the crystallization of PLA already mentioned. The reduction on the cold crystallization temperature was particularly marked in plasticized 3r-PLA-ATBC samples. When PLA crystallizes below 110 °C less stable crystals appears known as α' crystals [40]. From the cold crystallization peak in DSC thermogram it can be observed that disorder (crystals with α' form) to order (crystals with α form) phase transition takes place suggesting that a great fraction of the polymer is in an amorphous state due to the DSC cooling scan applied as it was already observed in plasticized PLA-ATBC samples [15, 41]. Somewhat increase on the T_{cc} values of KMN and KMW loaded films was observed with respect to r3-PLA-ATBC film, suggesting that somewhat less disordered crystals (α') are present in composite materials. Different crystallization degree was observed for PLA-ATBC-KMN-based films with respect to PLA-ATBC-KMW-based films. The higher crystallinity degree was found for those particles obtained from the fermentation of kombucha in yerba mate waste (KMW) and could be directly related with the better dispersion of KMW particles into the plasticized PLA-ATBC matrix, which are able to promote a higher nucleation effect [25].

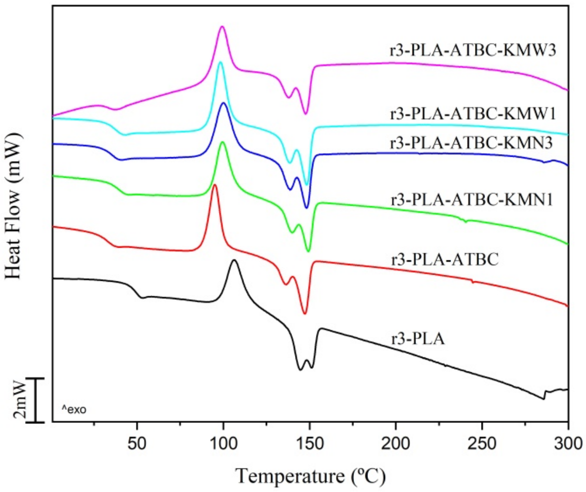

Figure 4. DSC second heating scan of 3r-PLA-ATBC based films.

Table 2. DSC thermal properties of 3r-PLA-ATBC based films.

Sample	T_g	T_{cc}	ΔH_{cc}	T_{mI}	T_{mII}	ΔH_m	χ_c

	(°C)	(°C)	(J g^{-1})	(°C)	(°C)	(J g^{-1})	(%)
r3-PLA	49.1	106.1	20.0	144.4	151.3	23.2	3.4
r3-PLA-ATBC	32.1	95.0	21.2	135.6	146.8	23.4	2.8
r3-PLA-ATBC-KMN1	39.1	99.5	19.2	139.7	148.9	19.8	0.7
r3-PLA-ATBC-KMN3	36.8	99.9	19.5	138.3	148.2	20.2	1.0
r3-PLA-ATBC-KMW1	37.3	98.4	20.3	137.7	148.1	22.8	3.1
r3-PLA-ATBC-KMW3	33.5	99.3	19.0	138.3	147.8	21.5	3.2

3.5. Thermogravimetric analysis

Thermal degradation parameters obtained by TGA are described in Table 3. Plasticization of PLA produced a decrease in $T_{5\%}$ and $T_{10\%}$, due to the decomposition of the plasticizer [42, 43] and a slight decrease in T_{max} was observed since the plasticization could make the polymer chains available to the thermal degradation as it was already observed on plasticized PLA with citrate esters [15, 22, 30]. The addition of 1% of KM increased $T_{5\%}$ and $T_{10\%}$ in the case of KMW, compared with plasticized r3-PLA. However, the addition of 3 wt.% of KM decreased $T_{5\%}$ and $T_{10\%}$ due to the low thermal stability of bacterial cellulose [44]. Regarding $T_{max,}$ it seemed that this value was enhanced by the addition of KMW and KMN at 1 wt.% since the value was close to the unplasticized r3-PLA film, but the addition of a higher amount of KM showed a significant decrease for 3 wt.% KMN but no modifications were observed for KMW. An overall conclusion for this study is that the addition of KMW enhanced the thermal properties of r3-PLA-ABTC, better than the KMN. This could be due to the higher crystallinity of r3-PLA-ABTC-KMW1 and r3-PLA-ABTC-KMW3 composites as showed DSC results.

Figure 5 shows the TGA (Figure 5-a) and DTG (Figure 5-b) curves. Thermal degradation of composites presented two steps of degradation. Firstly, the evaporation/degradation of the plasticizer overlaps with the initial degradation of KM, then in the second step of degradation PLA matrix was observed. For samples with higher concentrations of KM a higher percentage of mass was lost in the first step, confirming that in this event the KM was starting to degrade. It is important to note that the composites were thermally stable at processing temperatures usually used for PLA.

Table 3. TGA thermal properties of 3r-PLA-ATBC based films.

Sample	$T_{5\%}$ (°C)	$T_{10\%}$(°C)	T_{max} (°C)	Residual mass (%)
r3-PLA	296.1	313.52	354.4	0.4
r3-PLA-ATBC	217.4	272.28	351.1	0.8
r3-PLA-ATBC-KMN1	224.8	266.74	354.1	0.5
r3-PLA-ATBC-KMN3	214.7	246.21	344.7	0.7
r3-PLA-ATBC-KMW1	227.2	274.62	352.4	0.5
r3-PLA-ATBC-KMW3	208.1	243.19	351.8	0.5

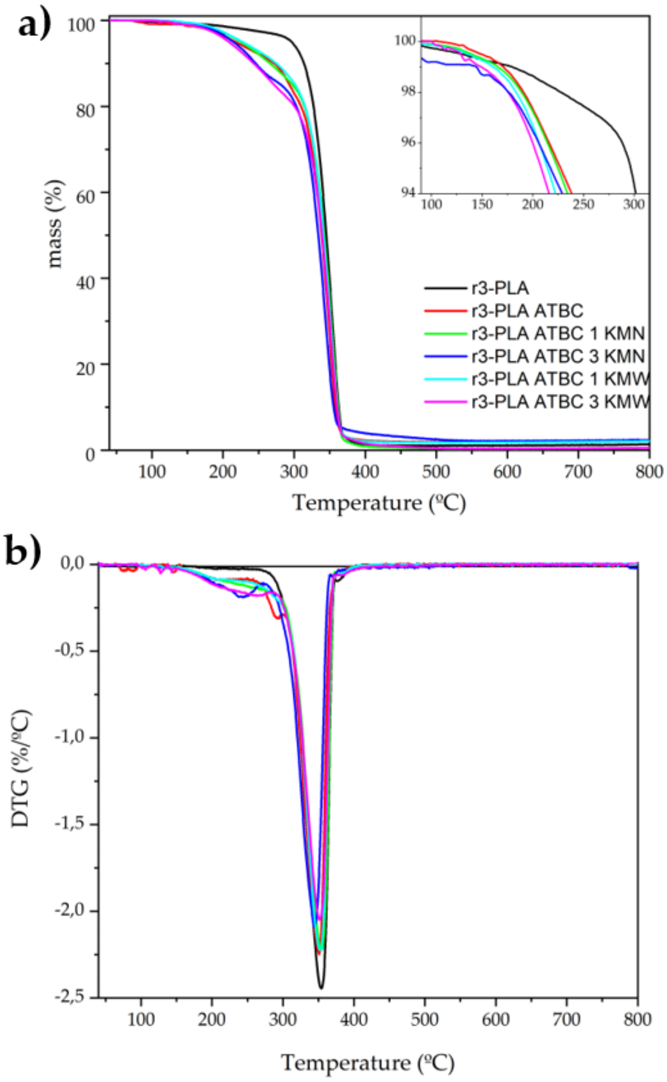

Figure 5. Dynamic TGA: a) and DTG b) of 3r-PLA-ATBC based films.

3.6. Tensile test

Mechanical performance of the r3-PLA and plasticized r3-PLA based films were analyzed by tensile test, which results in terms of Young Modulus (MPa), Tensile Strength (MPa) and Elongation at Break (%) are represented in Figure 6. Numerical values of these results are also gathered in Table 4. Firstly is worthy to note the decreased tensile parameters obtained for the here analyzed r3-PLA films processed by solvent casting compared with other works centered in PLA samples produced by melt extrusion [13]. As it was mentioned previously, this effect of the processing condition on the mechanical behavior of PLA films have been studied by Yang et al. [33], which relate this reduction of the tensile values, especially in terms of modulus, to the presence of captured residual chloroform which acts as a plasticizer. Otherwise, with the addition of ATBC (r3-PLA-ATBC) an enhanced in ductility is observed as was expected due to the proven effectiveness of this citrate ester as a PLA plasticizer [22, 25, 37, 45]. Specifically, r3-PLA-ATBC showed a notable modulus reduction with respect to r3-PLA, from ~1750 MPa to ~1550 MPa, as well as an increment in the elongation at break from 12% to 14%, while a very small drop in the tensile strength was obtained.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 December 2022 doi:10.20944/preprints202212.0344.v1

Table 4. Tensile test parameters values of 3r-PLA-ATBC based films.

Sample	E (MPa)	TS (MPa)	ε%
r3-PLA	1773.3 ± 164.1	27.7 ± 1.9	12.2 ± 1.4
r3-PLA-ATBC	1544.3 ± 154.3	26.5 ± 2.7	14.2 ± 1.5
r3-PLA-ATBC-KMN1	728.9 ± 88.7	15.4 ± 1.4	17.5 ± 0.4
r3-PLA-ATBC-KMN3	1319.5 ± 107.5	24.1 ± 2.4	5.4 ± 0.4
r3-PLA-ATBC-KMW1	826.1 ± 107.7	16.8 ± 1.5	10.9 ± 2.1
r3-PLA-ATBC-KMW3	487.6 ± 33.1	12.5 ± 2.2	13.5 ± 0.4

Regarding the addition of KM, at low content (1wt.%) both KMN and KMW induce a very similar effect in the modulus and tensile strength, showing r3-PLA-ATBC-KMN1 and r3-PLA-ATBC-KMN1 values around 750-850 MPa and 15-17 MPa respectively for these parameters. In composite materials when the particle dispersion is not homogenous enough the transfer load does not occur appropriately, which turned into a reduction of modulus and strength. This drawback of the introduction of cellulose derived particles in PLA films has been already reported by other author [46]. However, a significant difference between KMN and KMW was observed for the elongation at break results. In this sense, r3-PLA-ATBC-KMN1 showed a slight enhancement respected unloaded r3-PLA-ATBC (up to 17%) while the results of the r3-PLA-ATBC-KMW1 samples were closer to the non-plasticized r3-PLA. This difference in terms of ductility is totally correlated with the crystallinity values obtained in the thermal analysis. When the KM content was increased, the r3-PLA-ATBC film loaded with 3 wt.% of KMN showed a modulus and tensile strength somewhat higher, approaching to the r3-PLA-ATBC, while the elongation at break resulted lower. Lignocellulosic and cellulosic material have been widely studied as fillers of PLA based composites due to their high weight/strength ratio that can act as reinforcement when the interface contact area is adequate [47, 48]. Instead, with the addition of 3 wt.% of KMW that mechanical reinforcement does not occur properly, probably due to the more marked plasticizing effect produced by the KMW as a consequence of the possible degradation of some phenolic compounds (with less –OH able to establish hydrogen bonding interaction between then) present in pristine yerba mate due to the hydrothermal extraction process used to prepare the infusion that produced better interaction between PLA and ATBC allowing the higher elongation at break and in good accordance with the T_g value close to that of r3-PLA-ATBC (Table 2).

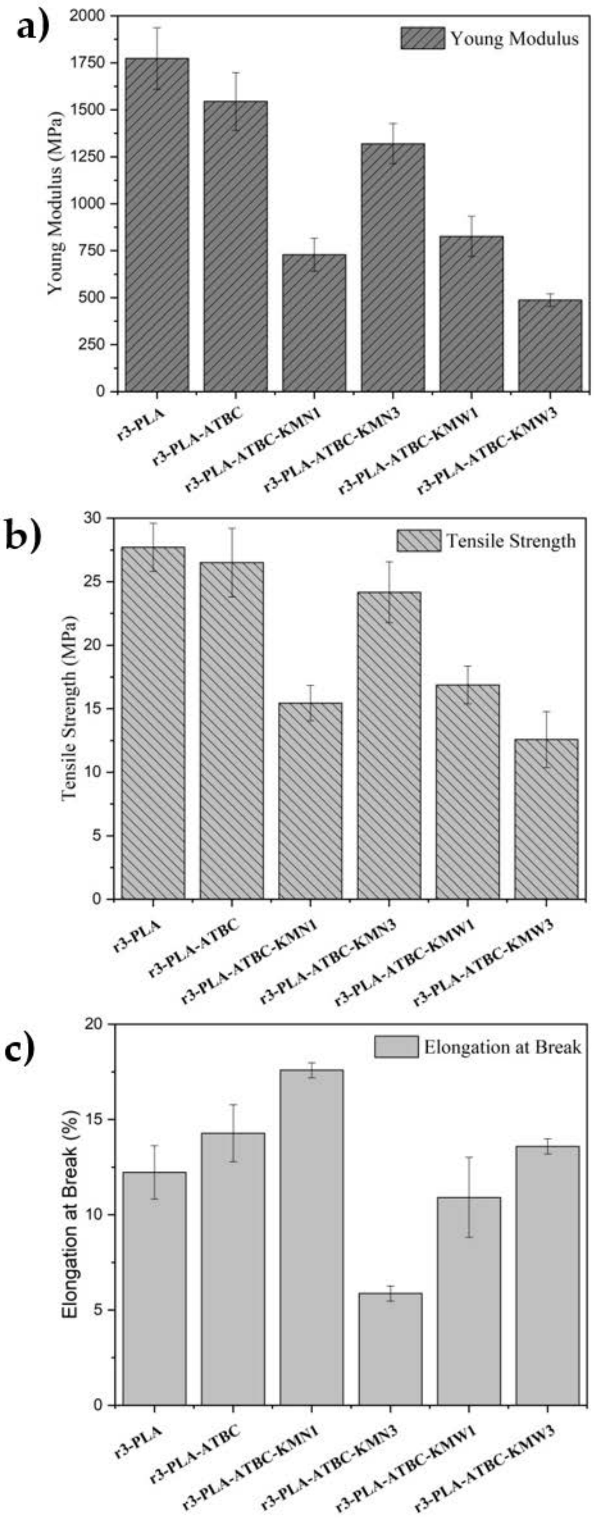

Figure 6. Tensile test measurements of 3r-PLA-ATBC based films.

3.7. Release studies and antioxidant ability

Specific migration tests were conducted to evaluate the potential antioxidant activity of the films, as microbial cellulose obtained from kombucha fermentation showed antioxidant properties concerning the tea used for its production [18]. In this work, kombucha was fermented in yerba mate sugared infusion and the antioxidant activity of yerba mate is well-known which mainly arises from its composition in phenolic compounds [19], even yerba mate waste is still able to provide antioxidant activity [15]. The radical scavenging activity (RSA) of each food simulant D1 sample after 10 contact days at 40 °C, considered by the current legislation the worst foreseeable conditions of the intended use [49], was determined by means of the DPPH method [41] and the results are shown in Figure 7.

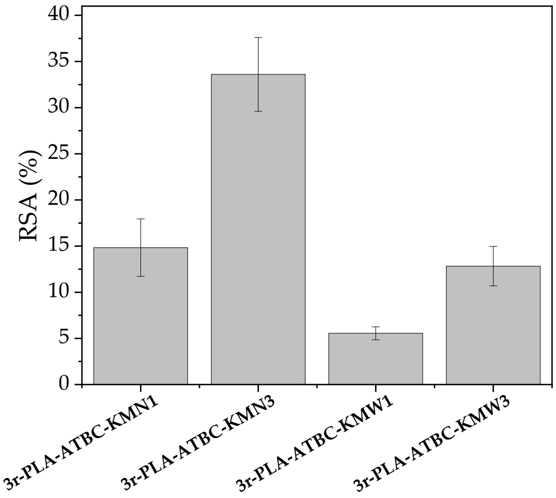

Figure 7. Radical scavenging activity of 3r-PLA-ATBC based films.

As expected, the r3-PLA film did not show any antioxidant activity. The KMN and KMW were processed into thin films and also subjected to the food simulant for comparison. The neat KMN film showed high RSA activity of 70.2± 1.1 % and 55.2± 11.7 %. Meanwhile, the films loaded with KMN or KMW showed somewhat antioxidant activity (as microbial cellulose obtained from kombucha fermentation possesses natural and remarkable antioxidant activity directly related to the infusion used for the fermentation [18]. Films loaded with KMN showed higher antioxidant activity than those loaded with KMW, as it is known that yerba mate waste possessed low content of polyphenol due to the hydrothermal extraction process produced during the infusion preparation (temperature higher than 80 °C) before obtaining the waste. The scavenging effect obtained here for 3r-PLA-ATBC-based materials loaded with microbial cellulose kombucha fermented in yerba mate or yerba mate waste (between 1 wt.% and 3 wt.%) are low, but it should be highlighted that they still possess somewhat antioxidant activity and, thus, resulting interesting for somewhat food crops or food packed protection. High antioxidant activity has been observed in materials containing yerba mate extract. For instance, Deladino et al. studied corn starch loaded materials with yerba mate extract in concentration of around 10 wt.% with respect to the starchy matrix, and found between 40 and 60 % of RSA [19]. The values obtained in the present work are in the range of other antioxidants materials based on tri-layer recycled PLA/sodium caseinate (SC)/recycled PLA-based materials reinforced with 1 and 3 wt.% of nanoparticles obtained from yerba mate waste (YMN) (RSA (%) of rPLA/SC/rPLA-YMN1 = 6.4 ± 0.1 and RSA (%) of rPLA/SC/rPLA-YMN3 = 11.0 ± 0.2) [39].

3.9. Water Contact Angle and Water Vapor Transmission Rate

The surface hydrophilic/hydrophobic properties of films were determined the measurement of static water contact angle (WCA) and the results are reported Figure 8-a. Meanwhile, the water vapor transmission rate (WVTR) values of plasticized r3-PLA-ATBC-based films are reported in Figure 8-b. The PLA film after 3 cycles of melt extrusion (3r-PLA) showed a water contact angle higher than 65 ° ascribed to hydrophobic surfaces (θ lower than 65 ° are ascribed to hydrophilic surfaces) [50]. Plasticized 3r-PLA sample (3r-PLA-ATBC) showed a lower WCA value, as was observed in already reported work of PLA and PLA-ATBC [25] as well as in agreement with the WVTR value in which r3-PLA andr3-PLA-ATBC showed similar WVTR properties, although being slightly higher the WVTR of 3r-PLA-ATBC. The plasticizing effect of ATBC into the r3-PLA matrix influenced the diffusion process as a consequence of the increased polymer chain mobility. The presence of either KMN or KMW in the 3r-PLA-ATBC matrix produced an increase of the surface wettabillity of the films (Figure 7-a) leading to values higher than 3r-PLA-ATBC and, thus, more hydrophobic. This unexpected increment of the hydrophobicity of the film surface even when hydrophilic cellulosic particles were added can be more related to the changes in the topographical properties as a consequence of the presence of cellulose particles. However, it should be highlighted that the composite films were still being more hydrophilic than 3r-PLA. The WVTR showed increased values with the presence of either KMN or KMW in the 3r-PLA-ATBC matrix (Figure 8-b), particularly in the case of KMN, probably due to the high amount of active compounds with –OH groups able to interact with water increasing the water diffusion through the film. Meanwhile, the KMW which showed less antioxidant activity (Figure 7) and consequently possess less amount of bioactive compounds within the polymeric matrix, allowed less water vapour transmission through the film.

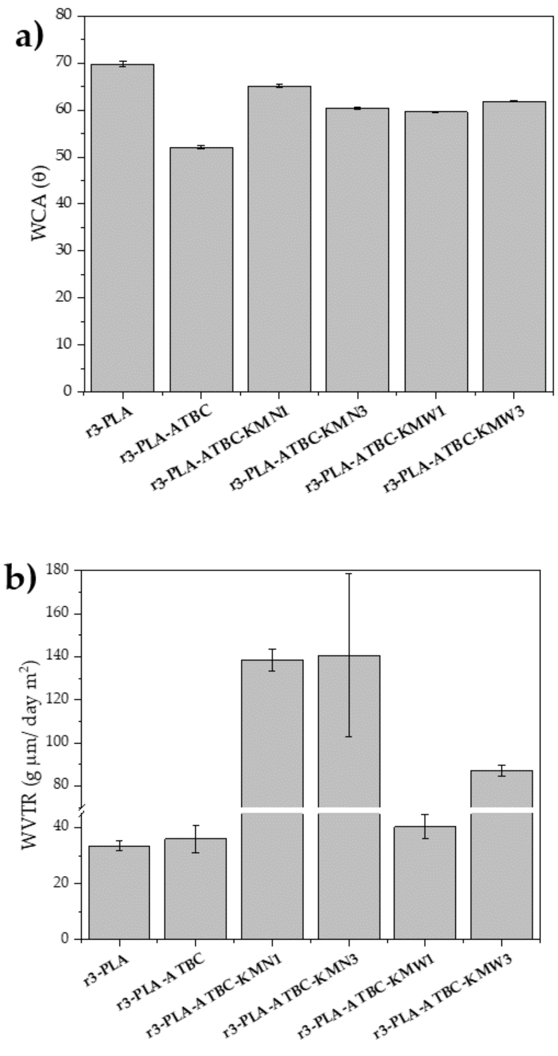

Figure 8. a) Static water contact angle measurements and b) water vapour transmission rate of 3r-PLA-ATBC based films.

4. Conclusions

Microbial cellulose particles were successfully obtained from kombucha beverage fermented in both infusion, pristine yerba mate and yerba mate waste, and were further used to reinforce plasticized PLA matrix subjected to three extrusion cycles (r3-PLA) aimed to simulate the revalorization of PLA from industrial PLA products rejected during the production line. The r3-PLA-based biocomposites reinforced with KMN and KMW were effectively prepared by solvent casting and the effect of yerba mate starting material (pristine or waste) and the amount added (1 and 3 wt.%) into the plasticized r3-PLA-ATBC was deeply investigated.

All films resulted optically transparent and FESEM micrographs revealed a good dispersion of microbial cellulose particles in the reprocessed polymeric matrix.

DSC analysis showed a crystallinity increase in r3-PLA-ATBC composites reinforced with KMW indicating that it was favoured the crystal growth and nucleation effect, while in tensile test measurements showed a more marked plasticization effect. Moreover, the materials reinforced with KMW showed less WVTR, showing improved barrier properties against water than the materials reinforced with pristine KMN. However, KMN reinforced r3-PLA-ATBC-KMN-based films showed higher antioxidant activity, but it should be highlighted that r3-PLA-ATBC-KMW-based films still showed antioxidant activity.

The reprocessed PLA (r3-PLA) in combination with ATBC and KM particles offers a promising perspective to produce transparent and flexible films with good water barrier, and mechanical properties that are suitable for antioxidant film for food packaging or agricultural mulch films.

Author Contributions: Conceptualization, M.M.F.G-S. and M.P.A.; methodology, A.A., D.L. and M.P.A.; software, A.A., E.C.P., D.L.; validation, A.A., E.C.P., S.S.A.M., D.L., M.M.F.G-S., M.A.P., R.B. and M.P.A.; formal analysis, A.A., E.C.P., S.S.A.M., D.L., M.M.F.G-S., M.A.P., M.P.A. ; investigation, A.A., E.C.P., S.S.A.M., D.L., M.M.F.G-S., M.A.P., R.B. and M.P.A.; resources, X.X.; data curation, A.A., E.C.P., S.S.A.M., D.L.,X.X.; writing—original draft preparation, A.A., D.L., M.M.F.G-S., M.A.P., M.P.A.; writing—review and editing, M.A.P., R.B. and M.P.A.; visualization, A.A., E.C.P., S.S.A.M., D.L., M.M.F.G-S., M.A.P., R.B. and M.P.A. ; supervision, M.M.F.G-S. and M.P.A.; project administration, M.M.F.G-S. and M.P.A.; funding acquisition, M.A.P., R.B. and M.P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministry of Science and Innovation (MICINN) through PID-AEI project (grants PID2020-116496RB-C22 and PID2021-123753NA-C32) and TED-AEI project (grants TED2021-129920A-C43 and TED2021-131762A-I00) funded by MCIN/AEI/10.13039/501100011033 and by ERDF “A way of making Europe” by the “European Union”. It was also funded by Comunidad de Madrid-CAM (“Ayudas de Estímulo a la Investigación de Jóvenes Doctores de la Universidad Politécnica de Madrid”, APOYO-JOVENES-21-VUI9G6-56-I7GABQ), Generalitat Valenciana-GVA grant numbers AICO/2021/025 and CIGE/2021/094 and National University of Quilmes (UNQ, Argentina) through the R&D program (Expediente 1300/19). Ph.D. Ángel Agüero and Ph.D. Diego Lascano acknowledge the Margarita Salas postdoctoral grants from the Ministerio de Universidades, Spain, funded by the European Union-Next Generation EU.

Institutional Review Board Statement: “Not applicable”

Data Availability Statement: All data is available through e-mail (m.arrieta@upm.es)

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Garcia-Garcia, D., A. Carbonell-Verdu, M. P. Arrieta, J. López-Martínez, and M. D. Samper. "Improvement of Pla Film Ductility by Plasticization with Epoxidized Karanja Oil." *Polymer Degradation and Stability* 179 (2020).
2. Auras, Rafael, Bruce Harte, and Susan Selke. "An Overview of Polylactides as Packaging Materials." *Macromolecular bioscience* 4, no. 9 (2004): 835-64.
3. Hernández-García, Eva, María Vargas, and Amparo Chiralt. "Effect of Active Phenolic Acids on Properties of Pla-Phbv Blend Films." *Food Packaging and Shelf Life* 33 (2022): 100894.
4. Arrieta, Marina Patricia, María Dolores Samper, Miguel Aldas, and Juan López. "On the Use of Pla-Phb Blends for Sustainable Food Packaging Applications." *Materials* 10, no. 9 (2017): 1008.
5. Arrieta, Marina P. "Influence of Plasticizers on the Compostability of Polylactic Acid." *Journal of Applied Research in Technology & Engineering* 2, no. 1 (2021): 1-9.
6. Gil Muñoz, Victor, Luisa M Muneta, Ruth Carrasco-Gallego, Juan de Juanes Marquez, and David Hidalgo-Carvajal. "Evaluation of the Circularity of Recycled Pla Filaments for 3d Printers." *Applied Sciences* 10, no. 24 (2020): 8967.

7. Beltrán, Freddys R, Marina P Arrieta, Diego Elena Antón, Antonio A Lozano-Pérez, José L Cenis, Gerald Gaspar, María U de la Orden, and Joaquín Martínez Urreaga. "Effect of Yerba Mate and Silk Fibroin Nanoparticles on the Migration Properties in Ethanolic Food Simulants and Composting Disintegrability of Recycled Pla Nanocomposites." *Polymers* 13, no. 12 (2021): 1925.
8. Cosate de Andrade, Marina F, Patricia Souza, Otávio Cavalett, and Ana R Morales. "Life Cycle Assessment of Poly (Lactic Acid)(Pla): Comparison between Chemical Recycling, Mechanical Recycling and Composting." *Journal of Polymers and the Environment* 24, no. 4 (2016): 372-84.
9. Dolores, S. M., A. M. Patricia, F. Santiago, and L. Juan. "Influence of Biodegradable Materials in the Recycled Polystyrene." *Journal of Applied Polymer Science* 131, no. 23 (2014).
10. Samper, M. D., D. Bertomeu, M. P. Arrieta, J. M. Ferri, and J. López-Martínez. "Interference of Biodegradable Plastics in the Polypropylene Recycling Process." *Materials* 11, no. 10 (2018).
11. Aldas, M., C. Pavon, H. De La Rosa-Ramírez, J. M. Ferri, D. Bertomeu, M. D. Samper, and J. López-Martínez. "The Impact of Biodegradable Plastics in the Properties of Recycled Polyethylene Terephthalate." *Journal of Polymers and the Environment* 29, no. 8 (2021): 2686-700.
12. Commission, European. "Commission Regulation (Eu) 2022/1616 of 15 September 2022 on Recycled Plastic Materials and Articles Intended to Come into Contact with Foods, and Repealing Regulation (Ec) No 282/2008 (Text with Eea Relevance)." 2022.
13. Agüero, Angel, Maria del Carmen Morcillo, Luis Quiles-Carrillo, Rafael Balart, Teodomiro Boronat, Diego Lascano, Sergio Torres-Giner, and Octavio Fenollar. "Study of the Influence of the Reprocessing Cycles on the Final Properties of Polylactide Pieces Obtained by Injection Molding." *Polymers* 11, no. 12 (2019): 1908.
14. Chariyachotilert, Chaiyatas, Satish Joshi, Susan EM Selke, and Rafael Auras. "Assessment of the Properties of Poly (L-Lactic Acid) Sheets Produced with Differing Amounts of Postconsumer Recycled Poly (L-Lactic Acid)." *Journal of Plastic Film & Sheeting* 28, no. 4 (2012): 314-35.
15. Arrieta, MP, Laura Peponi, Daniel López, and M Fernández-García. "Recovery of Yerba Mate (Ilex Paraguariensis) Residue for the Development of Pla-Based Bionanocomposite Films." *Industrial Crops and Products* 111 (2018): 317-28.
16. Tapias, Yuly A Ramírez, M Victoria Di Monte, Mercedes A Peltzer, and Andrés G Salvay. "Bacterial Cellulose Films Production by Kombucha Symbiotic Community Cultured on Different Herbal Infusions." *Food Chemistry* 372 (2022): 131346.
17. Ashrafi, Azam, Maryam Jokar, and Abdorreza Mohammadi Nafchi. "Preparation and Characterization of Biocomposite Film Based on Chitosan and Kombucha Tea as Active Food Packaging." *International Journal of Biological Macromolecules* 108 (2018): 444-54.
18. Ramírez Tapias, Yuly A, Mercedes A Peltzer, Juan F Delgado, and Andrés G Salvay. "Kombucha Tea by-Product as Source of Novel Materials: Formulation and Characterization of Films." *Food and Bioprocess Technology* 13, no. 7 (2020): 1166-80.
19. Deladino, Lorena, AS Teixeira, Alba S Navarro, I Alvarez, Antonio D Molina-García, and M Martino. "Corn Starch Systems as Carriers for Yerba Mate (Ilex Paraguariensis) Antioxidants." *Food and Bioproducts Processing* 94 (2015): 463-72.
20. (INYM), Instituto Nacional de la Yerba Mate. "Https://Inym.Org.Ar/Noticias/Estadisticas/79445-En-2020-El-Consumo-De-Yerba-Mate-Totalizo-311-7-Millones-De-Kilos.Html Consulted on 09/10/2022." (2022).
21. EFSA Panel of Food Contact Materials, Enzymes and Processing Aids. "Scientific Opinion on Flavouring Group Evaluation 10, Revision 3 (Fge. 10rev3): Aliphatic Primary and Secondary Saturated and Unsaturated Alcohols, Aldehydes, Acetals, Carboxylic Acids and Esters Containing an Additional Oxygenated Functional Group and Lactones from Chemical Groups 9, 13 and 30." *EFSA Journal* 10, no. 3 (2012): 2563.
22. Arrieta, Marina P, María D Samper, Juan López, and Alfonso Jiménez. "Combined Effect of Poly (Hydroxybutyrate) and Plasticizers on Polylactic Acid Properties for Film Intended for Food Packaging." *Journal of Polymers and the Environment* 22, no. 4 (2014): 460-70.
23. Arrieta, Marina Patricia, J López, D López, JM Kenny, and L Peponi. "Development of Flexible Materials Based on Plasticized Electrospun Pla–Phb Blends: Structural, Thermal, Mechanical and Disintegration Properties." *European Polymer Journal* 73 (2015): 433-46.
24. Van den Oever, MJA, B Beck, and J Müssig. "Agrofibre Reinforced Poly (Lactic Acid) Composites: Effect of Moisture on Degradation and Mechanical Properties." *Composites Part A: Applied Science and Manufacturing* 41, no. 11 (2010): 1628-35.
25. Arrieta, M. P., E. Fortunati, F. Dominici, J. López, and J. M. Kenny. "Bionanocomposite Films Based on Plasticized Pla-Phb/Cellulose Nanocrystal Blends." *Carbohydrate Polymers* 121 (2015): 265-75.
26. Turner, JFII, A Riga, Anne O'Connor, Jing Zhang, and Jennifer Collis. "Characterization of Drawn and Undrawn Poly-L-Lactide Films by Differential Scanning Calorimetry." *Journal of Thermal Analysis and Calorimetry* 75, no. 1 (2004): 257-68.
27. Trifol, Jon, Diana Catalina Marin Quintero, and Rosana Moriana. "Pine Cone Biorefinery: Integral Valorization of Residual Biomass into Lignocellulose Nanofibrils (Lcnf)-Reinforced Composites for Packaging." *ACS Sustainable Chemistry & Engineering* 9, no. 5 (2021): 2180-90.
28. European Commission. "No. 10, 2011 of 14, on Plastic Materials and Articles Intended to Come into Contact with Food " *Official Journal of European Union.* L12 January 2011 1 89. (2011).
29. Beltrán, Freddys R., Marina P. Arrieta, Gerald Gaspar, María U. de la Orden, and Joaquín Martínez Urreaga. "Effect of lignocellulosic Nanoparticles Extracted from Yerba Mate (Ilex Paraguariensis) on the Structural, Thermal, Optical and Barrier Properties of Mechanically Recycled Poly(Lactic Acid)." *Polymers* 12, no. 8 (2020): 1690.

30. García-Arroyo, P., M. P. Arrieta, D. Garcia-Garcia, R. Cuervo-Rodríguez, V. Fombuena, M. J. Mancheño, and J. L. Segura. "Plasticized Poly(Lactic Acid) Reinforced with Antioxidant Covalent Organic Frameworks (Cofs) as Novel Nanofillers Designed for Non-Migrating Active Packaging Applications." *Polymer* 196 (2020).
31. Garcia-Garcia, D., A. Carbonell-Verdu, M. P. Arrieta, J. López-Martínez, and M. D. Samper. "Improvement of Pla Film Ductility by Plasticization with Epoxidized Karanja Oil." *Polymer Degradation and Stability* 179 (2020): 109259.
32. Armentano, Ilaria, Elena Fortunati, Nuria Burgos, Franco Dominici, Francesca Luzi, Stefano Fiori, Alfonso Jiménez, Kicheol Yoon, Jisoo Ahn, and Sangmi Kang. "Processing and Characterization of Plasticized Pla/Phb Blends for Biodegradable Multiphase Systems." (2015).
33. Yang, W., E. Fortunati, F. Dominici, J. M. Kenny, and D. Puglia. "Effect of Processing Conditions and Lignin Content on Thermal, Mechanical and Degradative Behavior of Lignin Nanoparticles/Polylactic (Acid) Bionanocomposites Prepared by Melt Extrusion and Solvent Casting." *European Polymer Journal* 71 (2015): 126-39.
34. Burgos, Nuria, Verónica P. Martino, and Alfonso Jiménez. "Characterization and Ageing Study of Poly(Lactic Acid) Films Plasticized with Oligomeric Lactic Acid." *Polymer Degradation and Stability* 98, no. 2 (2013): 651-58.
35. Arrieta, Marina P., Miguel Perdiguero, Stefano Fiori, José María Kenny, and Laura Peponi. "Biodegradable Electrospun Pla-Phb Fibers Plasticized with Oligomeric Lactic Acid." *Polymer Degradation and Stability* 179 (2020): 109226.
36. Labrecque, LV, RA Kumar, V Dave, RA Gross, and SP McCarthy. "Citrate Esters as Plasticizers for Poly (Lactic Acid)." *Journal of Applied Polymer Science* 66, no. 8 (1997): 1507-13.
37. Courgneau, Cécile, Violette Ducruet, Luc Avérous, Jean Grenet, and Sandra Domenek. "Nonisothermal Crystallization Kinetics of Poly (Lactide)—Effect of Plasticizers and Nucleating Agent." *Polymer Engineering & Science* 53, no. 5 (2013): 1085-98.
38. Beltrán, F. R., V. Lorenzo, J. Acosta, M. U. de la Orden, and J. Martínez Urreaga. "Effect of Simulated Mechanical Recycling Processes on the Structure and Properties of Poly(Lactic Acid)." *Journal of Environmental Management* 216 (2018): 25-31.
39. Arrieta, Marina Patricia, Freddys Beltran, Sara Soledad Abarca de las Muelas, Gerald Gaspar, Rafael Sanchez Hernandez, Maria Ulagares de la Orden, and Joaquin Martinez Urreaga. "Development of Tri-Layer Antioxidant Packaging Systems Based on Recycled Pla/Sodium Caseinate/Recycled Pla Reinforced with Lignocellulosic Nanoparticles Extracted from Yerba Mate Waste." *Express Polymer Letters* 16, no. 8 (2022): 881-900.
40. Zhang, Jianming, Kohji Tashiro, Hideto Tsuji, and Abraham J Domb. "Disorder-to-Order Phase Transition and Multiple Melting Behavior of Poly (L-Lactide) Investigated by Simultaneous Measurements of Waxd and Dsc." *Macromolecules* 41, no. 4 (2008): 1352-57.
41. Arrieta, Marina Patricia, Maria del Mar Castro-Lopez, Emilio Rayón, Luis Fernando Barral-Losada, José Manuel López-Vilariño, Juan López, and María Victoria González-Rodríguez. "Plasticized Poly (Lactic Acid)–Poly (Hydroxybutyrate)(Pla–Phb) Blends Incorporated with Catechin Intended for Active Food-Packaging Applications." *Journal of agricultural and food chemistry* 62, no. 41 (2014): 10170-80.
42. Tee, Yee Bond, Rosnita A Talib, Khalina Abdan, Nyuk Ling Chin, Roseliza Kadir Basha, and Khairul Faezah Md Yunos. "Comparative Study of Chemical, Mechanical, Thermal, and Barrier Properties of Poly (Lactic Acid) Plasticized with Epoxidized Soybean Oil and Epoxidized Palm Oil." *BioResources* 11, no. 1 (2016): 1518-40.
43. Arrieta, M. P., J. López, S. Ferrándiz, and M. A. Peltzer. "Characterization of Pla-Limonene Blends for Food Packaging Applications." *Polymer Testing* 32, no. 4 (2013): 760-68.
44. Threepopnatkul, Poonsub, Amnard Sittattrakul, Karn Supawititpattana, Panat Jittiarpon, Phumin Raksawat, and Chanin Kulsetthanchalee. "Effect of Bacterial Cellulose on Properties of Poly (Lactic Acid)." *Materials Today: Proceedings* 4, no. 5 (2017): 6605-14.
45. Maiza, Mounira, Mohamed Tahar Benaniba, Guilhem Quintard, and Valerie Massardier-Nageotte. "Biobased Additive Plasticizing Polylactic Acid (Pla)." *Polimeros* 25 (2015): 581-90.
46. Geng, Shiyu, Jiayuan Wei, Yvonne Aitomäki, Maxime Noël, and Kristiina Oksman. "Well-Dispersed Cellulose Nanocrystals in Hydrophobic Polymers by in Situ Polymerization for Synthesizing Highly Reinforced Bio-Nanocomposites." *Nanoscale* 10, no. 25 (2018): 11797-807.
47. Iwatake, Atsuhiro, Masaya Nogi, and Hiroyuki Yano. "Cellulose Nanofiber-Reinforced Polylactic Acid." *Composites Science and Technology* 68, no. 9 (2008): 2103-06.
48. Luzi, F., L. Torre, J. M. Kenny, and D. Puglia. "Bio- and Fossil-Based Polymeric Blends and Nanocomposites for Packaging: Structure-Property Relationship." *Materials* 12, no. 3 (2019).
49. Commission, European. "Commission Regulation (Eu) No. 10, 2011 of 14, on Plastic Materials and Articles Intended to Come into Contact with Food " *Official Journal of the European Union* L12/1 (2011): 1-89.
50. Hambleton, Alicia, Maria-José Fabra, Frédéric Debeaufort, Cécile Dury-Brun, and Andrée Voilley. "Interface and Aroma Barrier Properties of Iota-Carrageenan Emulsion–Based Films Used for Encapsulation of Active Food Compounds." *Journal of food engineering* 93, no. 1 (2009): 80-88.