

Green Composites from Residual Microalgae Biomass and Poly(butylene adipate-co-terephthalate): Processing and Plasticization

Simonet Torres,^{*,†,‡,⊥} Rodrigo Navia,^{‡,§} Rachel Campbell Murdy,[⊥] Peter Cooke,^{||} Manjusri Misra,^{⊥,⊗} and Amar K. Mohanty^{⊥,⊗}

[†]Desert Bioenergy S.A., Av. Presidente Kennedy 5146, Las Condes, Santiago 7550000, Chile

[‡]Departamento de Ingeniería Química y Núcleo Científico Tecnológico en Biorecursos, Universidad de La Frontera, Casilla 54-D, Temuco 4811230, Chile

[§]Centre for Biotechnology and Bioengineering, Universidad de La Frontera, Casilla 54-D, Temuco 4811230, Chile

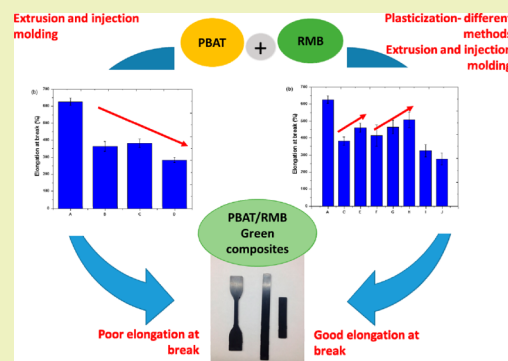
[⊥]Bioproducts Discovery and Development Centre, Department of Plant Agriculture, University of Guelph, Crop Science Building, Guelph, Ontario N1G 2W1, Canada

^{||}Core University Research Resources Laboratory, New Mexico State University, 945 College Drive, Skeen Hall, Las Cruces, New Mexico 88003, United States

[⊗]School of Engineering, University of Guelph, Thornbrough Building, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: Innovative biocomposites from residual microalgae biomass (RMB), a byproduct of biodiesel production, and PBAT (poly(butylene adipate-co-terephthalate)) have been prepared in this study. RMB was characterized by Fourier transform infrared spectroscopy (FT-IR) and its thermal stability was determined. Subsequently, RMB and PBAT biocomposites were prepared by extrusion and injection molding. Incorporation of 10, 20 and 30% RMB in the biocomposites was studied. The biocomposites were characterized using FT-IR and thermogravimetric analysis, and their mechanical properties were compared, including tensile, flexural and impact strength. The effect of RMB on the morphology of the polymer matrix was analyzed by scanning electron microscopy and confocal laser scanning microscopy. RMB plasticization was performed with glycerol and urea, comparing different proportions of glycerol and urea. The studies show that it is possible to use RMB in the manufacture of biocomposites with PBAT, obtaining the best extrusion results with 20% RMB. Optimal result was achieved with 30% glycerol and 7.5 phr of urea.

KEYWORDS: Biocomposites, microalgae biomass, biodiesel, poly(butylene adipate-co-terephthalate), plasticizers, glycerol, urea



INTRODUCTION

Increased worldwide demand for energy, depletion of fossil fuels, greenhouse gas emissions and global warming are ongoing problems faced by humanity. Over the past decade, scientific studies have focused on exploring new energy sources that are more environmentally friendly, renewable and sustainable. One promising energy alternative to the use of petrodiesel is biodiesel.¹

Biodiesel consists of series of monoalkyl esters of fatty acids derived from renewable biomass. The main source of raw material for biodiesel production is vegetable oil,² but the disadvantage of this source is the need for large tracts of fertile land for cultivation; this in turn has led to environmental problems arising from deforestation of tropical regions. Some have proposed the use of inedible oils, such as *Jatropha curcas* oils that do not require fertile land, but this has not been commercially viable due to the high costs of the raw material.³ Other alternatives for biodiesel production are animal fat and

oil for frying foods (used cooking oil) but these involve a high cost to refine the product.^{4,5} Although there is a large variety of raw materials that can be used to generate biodiesel and eliminate our dependence on petroleum, the reality is that none of these raw materials can satisfy global demand for petrodiesel. According to Chisti et al. (2007),⁶ 0.53 billion m³ of biodiesel per year would be needed to replace all of the fuel consumed in the United States (at the current rate of consumption). Therefore, these sources are insufficient to meet current and future worldwide demand for biodiesel.

Today, microalgae have emerged as a promising alternative for third-generation biodiesel production. Microalgae are photosynthetic organisms that consume carbon dioxide, transforming it into lipids. The advantages of microalgae as

Received: November 25, 2014

Revised: February 19, 2015

Published: February 26, 2015

lipid producers for biodiesel production compared to other sources include their rapid growth, high lipid content, ability to fix carbon dioxide and their ability to grow in either fresh water, saltwater or wastewater.⁷ Moreover, cultivation of microalgae does not require agricultural land. Over the past decade, scientific interest has focused on biodiesel generation from microalgae and the diverse alternatives for developing a competitive process compared to petrodiesel.^{8–11} According to Chisti et al. (2007),⁶ microalgae (with a lipids content of 30% dry mass) yields 58.700 L oil/Ha. This yield is approximately 10 times higher than that of palm oil (5950 L/Ha) and 100 times higher than soybean oil (446 L/Ha). Biodiesel from microalgae is not yet commercially available; the scale of production of microalgae biodiesel must increase to become competitive with petrodiesel and biodiesel from other sources.

There are several processes used to obtain biodiesel from microalgae. Some authors mention that it is necessary to extract the microalgae oil and use this oil in a secondary transesterification process. Another alternative is to directly transesterify microalgae biomass with a methylation agent and catalyst.^{12–14} Both processes generate a depleted biomass waste, otherwise known as residual microalgae biomass (RMB). It is postulated that a commercial scale-up of microalgae biodiesel production could generate high quantities of RMB, as this residual biomass contains high quantities of protein, carbohydrates, fiber and ash.¹⁵ Considering the chemical composition of RMB, this coproduct could be used as animal feed but would compete with residuals from ethanol production such as distiller's dried grains with solubles (DGGs) and other sources of animal feed. New applications of RMB must be found to generate an environmentally friendly and sustainable alternative for the production of microalgae biodiesel to generate additional economic return.

Today, several raw materials containing cellulose and hemicellulose are produced worldwide. These raw materials include natural fibers from agro-industry as well as wastes and coproducts from the food and the biofuels industry. Composites with natural fibers have gained market due to their low cost, availability, degradability and contribution to increase the resistance of the fabricated materials. In this regard, one of the most interesting and widely available additives for biocomposites are dried distiller's grains with solubles (DDGS) from bioethanol industry. Although biodiesel production from microalgae is still at pilot scale, a real alternative is needed for the use of microalgae residual biomass if the industry moves to large-scale.^{16,17}

The application of industrial or agroindustrial wastes as additives for fabricating composites has generated a great deal of interest in the scientific community due to its positive environmental effects, including: (i) a reduced carbon footprint, (ii) waste material management, (iii) production of biodegradable products and (iv) creation of products with biodegradable polymers at a lower cost. Studies in which different additives have been used in the fabrication of biocomposites applied to different polymer matrices are indicative of the ongoing research activities in this area.^{16–23} Although additives can be used in the preparation of composites, the majority of these fillers are hydrophilic, which are not compatible with hydrophobic polymers, thus necessitating compatibilization to improve the mechanical properties of the resulting biocomposites. There are many options for compatibilization, depending on the additive and matrix being used, for example, silane

treatment,^{24,25} plasticization with glycerol^{26,27} and polymer grafting.^{28,29} One alternative for generating sustainable biodiesel production is the application of RMB in biocomposites. Jang et al. (2013)³⁰ used marine algae biomass to fabricate biocomposites with polypropylene (PP). They also used biomass from pretreated microalgae, simulating waste from the energy industry. The authors mention that green algae pretreated with sulfuric acid can be used as an additive with PP. In addition, biocomposites of RMB and poly(butylene succinate) (PBS) have been prepared by extrusion and injection molding, including the characterization of biocomposites fabricated with 10 and 20% RMB.¹⁵ Treatment of residual microalgal biomass (RMB) with silanes has been studied in an attempt to improve its mechanical properties.³¹ Given the nature of RMB, another alternative is plasticization. Plasticization is a process usually applied to additives rich in protein such as soybean meal, as well as to polysaccharides such as starch. For protein-rich materials, the most commonly used plasticizers are water, urea and glycerol. These materials have generally a low molecular weight, so they are highly mobile and can organize themselves three-dimensionally in the polymer chain. There are at least three theories about the behavior of plasticizers in polymeric materials: (1) the lubricity theory, (2) the gel theory and (3) the free volume theory. According to these theories, plasticizers act facilitating the mobility of the molecules, breaking intermolecular forces polymer–polymer.^{32,33}

The objective of this study was to fabricate extruded and injection molded biocomposites incorporating RMB with poly(butylene adipate-*co*-terephthalate) (PBAT). The inclusion of varying quantities of RMB (10, 20 and 30%) in the polymer matrix was analyzed in the extrusion process. In addition, the plasticization of RMB and PBAT with glycerol and urea was studied, including the effect on the mechanical, thermal and dynamic properties of biocomposites with increasing RMB content, as well as the effect of compatibilization with glycerol and urea in biocomposites using an RMB content of 20% by weight. The effects on the morphology of the polymer matrix due to an increase in RMB content were analyzed using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

■ EXPERIMENTAL SECTION

Materials. Poly(butylene adipate-*co*-terephthalate) (PBAT) pellets were obtained from Zhejiang Hangzhou Xinfu Pharmaceutical Co. Ltd. The microalgae used in this work, *Nannochloropsis gaditana*, were cultivated by Desert Bioenergy S.A. at the University of Antofagasta in Antofagasta, Chile. Glycerol (99.5%) and urea (99.5%, for analysis) were obtained from Fischer Scientific. NaOH (for analysis) pellets were obtained from Merck S.A.

Microalgae Biomass. Microalgae were grown in raceway ponds in Northern Chile in a semicontinuous cultivation regime. The concentration of microalgae in the cultivation raceways ranged between 0.5 and 0.1 g/L. The biomass was afterward concentrated using centrifugation to obtain a paste with a solids content of up to 20%. The concentrated biomass was dried at 50 °C for 48 h. The dry biomass was then used to produce biodiesel through direct transesterification and to obtain the residual microalgae biomass (RMB). The RMB was neutralized with NaOH (1 M) and dried for subsequent analysis. The chemical profile of microalgae biomass and RMB was determined by proximate analysis according to the Association of Analytical Communities (AOAC, Official Methods).

Preparation of Biocomposites Using RMB. PBAT and RMB were dried at 80 °C before being used to prepare the biocomposites. The composition of the composites was fixed at specific PBAT/RMB

w/w ratios (90/10; 80/20; 70/30). Processing experiments were carried out in a micro twin-screw extruder (DSM Research, The Netherlands). The extrusion temperature was 140 °C with a residence time of 2 min at 100 rpm. The mixture was collected in the injection molding system at 30 °C (DSM Research, The Netherlands).

Plasticization of RMB with Glycerol and Urea. Dry Process. RMB was dried at 60 °C, and further premixed with using a kitchen mixer (KitchenAid K45SSWH Classic, USA). Glycerol content of 20% and 30% by weight was used (Table 1). Once the glycerol was mixed

Table 1. Sample Reference for Plasticized RMB Used in This Study (Preformulate RMB)

sample	plasticized RMB			
	RMB (wt %)	glycerol (wt %)	water (phr)	urea (phr)
RMB-p1 ^a	80	20	0	0
RMB-p2 ^a	70	30	0	0
RMB-p3 ^b	70	30	10	0
RMB-p4 ^b	70	30	10	7.5
RMB-p5 ^b	70	30	10	15
RMB-p6 ^b	70	30	10	30

^aDry process. ^bWet process.

with the biomass, it was allowed to rest in a plastic bag for 3 h. The mixture was processed in the extruder at 100 °C to generate the plasticized biomass (preformulate RMB: RMB-p1 and RMB-p2).

Wet Process. RMB (70%) was combined in a kitchen mixer with glycerol (30%), water (10 phr) and urea (Table 1). The obtained mixture was stored overnight in a plastic bag. The mixture was then processed in the extruder at 100 °C obtaining a plasticized RMB (preformulate RMB: RMB-p3 to RMB p-6). RMB-p3 to RMB p-6 was subsequently used in the formulation of biocomposites with PBAT.

Preparation of Biocomposites Using RMB with Plasticizers (RMB-p). PBAT and RMB pellets were dried at 80 °C before being used to prepare the biocomposites. The formulation of the biocomposites is shown in Table 2, with an RMB composition of 20% for comparison with PBAT/RMB (80/20). Processing experiments were carried out as described above.

Fourier Transform Infrared Spectroscopy (FT-IR). RMB and all composites were characterized by FT-IR spectroscopy. In this work, a Thermo Scientific Nicolet 6700 FT-IR spectrometer (USA) with an ATR (attenuated total reflectance) attachment was used to obtain all FT-IR spectra. The FT-IR spectra of the composites were recorded in the 4000–500 cm⁻¹ region at room temperature, with 64 scan and resolution of 4 cm⁻¹.

Mechanical Tests. The tensile and flexural properties of neat PBAT and all composites were tested using a universal testing machine (Instron model 3382) (Massachusetts, USA). The tensile test was carried out according to ASTM Standard D638, with a length of 50 mm and a cross-head speed of 50 mm min⁻¹. The mold had a type IV

shape. The specimen dimension was 116 × 6 × 2 mm and was prepared within a deviation of ±5%. The flexural test was determined according to ASTM Standard D790, with a cross-head speed of 14 mm min⁻¹. The specimen dimension was 126 × 12 × 3 mm and was prepared within a deviation of ±5%. The specimen was tested flatwise on a support span, resulting in a support span-to-depth ratio of 16. Flexural test was stopped when the sample reached the 5% deflection or sample was broken before 5%. The impact strength was tested with a testing machine (Testing Machines Inc., Delaware, USA), according to ASTM Standard D256.

Density. An Alfa Mirage (Osaka, Japan) electronic densimeter MD-300 was used to obtain the densities of all composites. Triplicate samples were tested for statistical inference.

Thermogravimetric Analysis (TGA). The thermal stability of RMB and the composites was determined using a thermogravimetric analyzer (TGA Q500, TA Instruments, Inc.) (Delaware, USA). The measurements were obtained at a heating rate of 10 °C min⁻¹ from room temperature to 600 °C, under a nitrogen atmosphere. The TGA results and the derivative TGA curves (DTGA) were analyzed with TA Instruments software.

Dynamic Mechanical Analysis (DMA). The storage modulus and tan delta of neat PBAT and PBAT/RMB composites was determined using the DMA Q800 (TA Instruments, Inc.) (Delaware, USA). The experiments were measured from -50 to +100 °C, with a dual cantilever clamp and rate temperature of 3 °C min⁻¹. The specimen dimension was 63 × 12.7 × 3.2 mm and was prepared within a deviation of ±5%.

Morphological Characterization. Scanning Electron Microscopy (SEM). Morphological studies of cryofractured samples, tensile fracture surfaces and transverse cutting surfaces were observed using scanning electron microscopy (SEM) (Inspect S50, FEI, The Netherlands). The samples were sputter coated with gold for 60 s using a Cressington sputter coater 108 auto (Cressington Scientific Instruments Inc., UK) under an argon atmosphere. An accelerating voltage of 20 kV was used for imaging, the coating current prior to SEM was 30 mA and the deposition 15 nm/min. Samples were viewed at magnifications of 500×, 1000×, 2500× and 5000×.

Confocal Laser Scanning Microscopy (CLSM). Fluorescence images of sample surfaces were collected as two emission channels (500–580 and 660–690 nm) into stacks of optical sections approximately 20–30 μm thick and overlaid into a single, extended focus image using confocal laser scanning microscopy (CLSM) (model TCS SP5 II, Leica Microsystems, Germany).

RESULTS AND DISCUSSION

Chemical Characterization and Infrared Spectroscopy of Microalgae Biomass and RMB. The chemical composition of the microalgae and the RMB are provided in Table 3. According to these results, there is a significant difference in the composition of the microalgae and the RMB. Although the

Table 2. Formulation, Mechanical Properties, Density and HDT Data for Composites Prepared in This Work

sample	formulation of all composites	flexural strength (MPa)	flexural modulus (MPa)	impact strength (Jm ⁻¹)	density (g cm ⁻³)	HDT ^b (°C)
A	PBAT/RMB (100/0)	4.1 ± 0.1	91.9 ± 13.4	N.B	1.21	41.2 ± 0.4
B	PBAT/RMB (90/10)	4.8 ± 0.2	113.1 ± 11.7	N.B	1.24	41.6 ± 1.5
C	PBAT/RMB (80/20)	5.6 ± 0.2	129.0 ± 11.5	N.B	1.26	41.6 ± 1.3
D	PBAT/RMB (70/30)	5.7 ± 0.4	151.0 ± 20.3	199.2 ± 10.5	1.28	41.2 ± 0.8
E	PBAT/RMB-p1(76/24) ^a	3.5 ± 0.4	104.2 ± 50.0	N.B	1.25	37.7 ± 1.0
F	PBAT/RMB-p2 (73/24) ^a	4.2 ± 0.2	86.74 ± 7.4	N.B	1.26	37.2 ± 0.8
G	PBAT/RMB-p3 (73/24) ^a	4.3 ± 0.2	102.0 ± 5.9	N.B	1.25	
H	PBAT/RMB-p4 (72/28) ^a	3.2 ± 0.1	107.4 ± 25.6	N.B	1.25	
I	PBAT/RMB-p5 (71/29) ^a	5.3 ± 2.4	122.3 ± 19.1	N.B	1.23	
J	PBAT/RMB-p6 (68/32) ^a	4.2 ± 0.4	120.0 ± 25.4	N.B	1.23	

^aIn these composites, the weight ratio of dry RMB was 20% compared to the effect of plasticizer with PBAT/RMB (80/20). ^bHDT: heat deflection temperature.

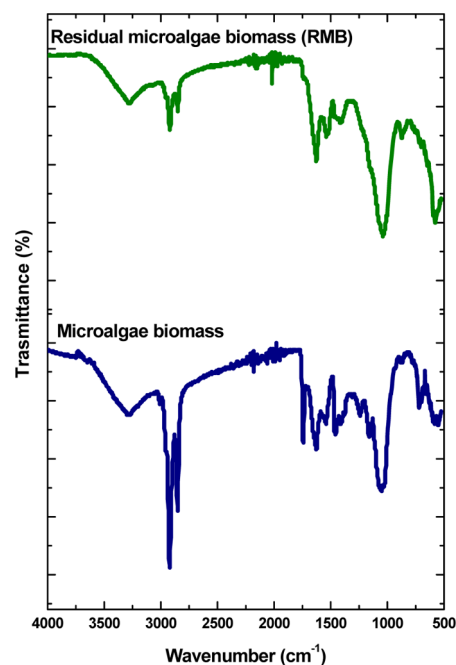
Table 3. Chemical Composition of *Nannocloropsis gaditana* (Microalgae Biomass), before and after Direct Transesterification Reaction

constituent (%)	microalgae biomass ^a	residual microalgae biomass (RMB) ^b
protein	23	42
fat	33	7
ash	22	37
fiber + free carbohydrates	22	14

^aPercentage before direct transesterification reaction. ^bPercentage after direct transesterification reaction.

microalgae biomass is rich in protein and oil, RMB contains high amounts of protein and ash, with a small percentage of oil and fiber. Similar results were obtained by Toro et al. (2013)¹⁵ with RMB obtained after extracting oil and protein. In this study, direct transesterification was used to produce biodiesel from microalgae biomass (due to its high oil content) and this in turn reduced the oil content of the RMB. The RMB's protein content increased because it was not extracted in the direct transesterification process, resulting in a coproduct (RMB) that is rich in protein and can be used in other applications such as biocomposites.

Table 4 shows the assignment of the major peaks in the infrared spectrum for the microalgae biomass and the RMB. The FT-IR spectrum (Figure 1) of the microalgae revealed 4 bands in the region of 3600–1600 cm⁻¹: a wide, weak band centered around 3278 cm⁻¹ corresponding to a small amount of moisture present in the sample; a strong signal at 2921 cm⁻¹ corresponding to the CH₂ in a methylene group; a moderate band at 2852 cm⁻¹ that was assigned to C–H stretching vibrations of the methyl and methylene group; a weak signal at 1743 cm⁻¹ corresponding to lipid esters and fatty acids. The latter three bands correspond to the lipid fraction of the microalgae biomass. The bands at 1625 and 1540 cm⁻¹ correspond to N–H or C=O stretching (amide I) and N–H bending (amide II) for the protein group, respectively.²⁶ In addition, a band at 1054 cm⁻¹ may be C–N bonds for proteins and C–O–C stretching for polysaccharides. The FT-IR spectrum of the RMB revealed only three bands in the region of 3600–1600 cm⁻¹: a weak band at 3228 cm⁻¹ corresponding to moisture, and two weak bands at 2017 and at 2850 cm⁻¹ due to the CH₂ of a methylene group and C–H stretching of methyl

**Figure 1.** FT-IR spectra of initial microalgae biomass and residual microalgae biomass (RMB).

and methylene groups, respectively. The FT-IR spectrum did not show the band at 1743 cm⁻¹ that corresponds to fatty acids. These results are consistent with the chemical characterization of RMB, presenting a low lipid content. The region of 1700–1000 cm⁻¹ on the RMB spectrum is similar to the spectrum of the microalgae biomass. The bands corresponding to the N–H or C–O stretching (amide I) and N–H bending (amide II) for the protein group are present.^{34,35}

The FT-IR results and proximate analysis contribute to understand the changes in chemical composition of microalgae biomass, as in the biodiesel production process, high temperatures and concentrated acid are used to improve microalgae lipids conversion yield. These aggressive reaction conditions generate a residual microalgae biomass with a very different composition compared to the initial one. Initially, the biomass has a high oil content, which is extracted during the biodiesel production process, remaining in the RMB only a 7% oil. The FT-IR results (Table 4) show the signs of fatty acids in

Table 4. FT-IR Assignment for Microalgae Biomass and RMB^a

wavenumber (cm ⁻¹)		assignment	functional groups
MB	RMB		
3278 (w)	3228 (w)		moisture
2921 (s)	2917 (w)	ν CH ₂	CH ₂ methylene group
2852 (s)	2850 (w)	ν CH ₂ , CH ₃	CH ₂ , CH ₃ methyl and methylene group
1743 (m)	Np	ν C=O	ester of lipids and fatty acids
1625 (m)	1627 (m)	ν C=O (amide I)	protein
1540 (w)	1538	δ N–H (amide II)	protein
1455 (m)	1409 (m)	ν CH ₂ , CH ₃	CH ₂ , CH ₃ methyl and methylene group
1415 (m)			
1240 (w)	Np	ν C–O	ester of lipids and fatty acids
1157 (w)			
1054 (m)	1041 (s)	ν (C–O–C) C–N bonds	polysaccharides protein

^aw, weak; m, moderate; s, strong; Np, not present.

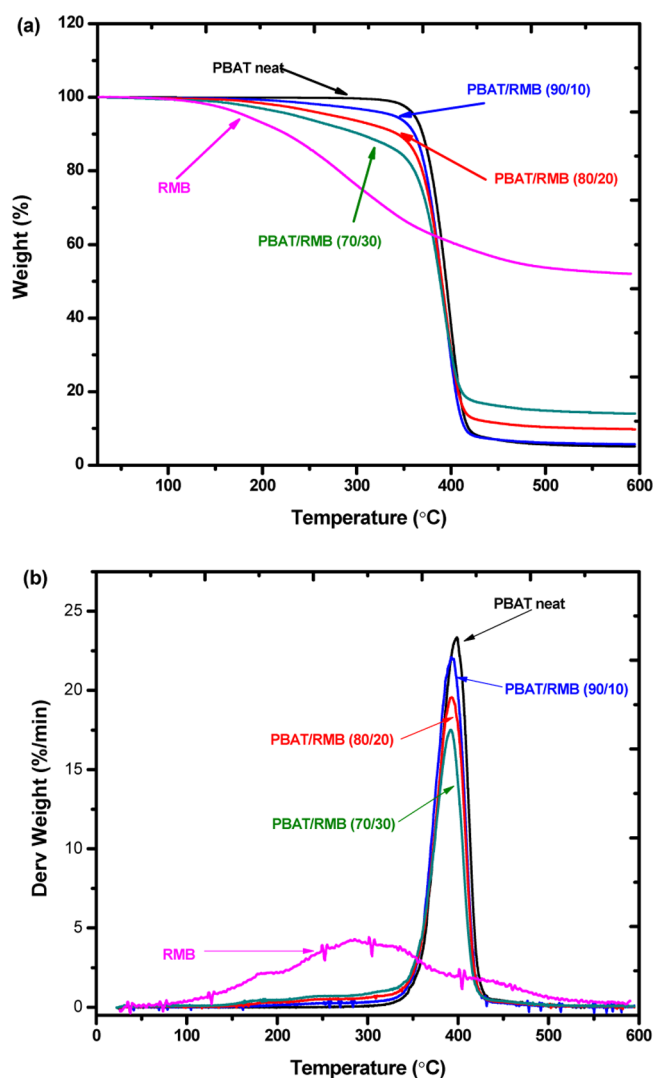


Figure 2. TGA and DTGA curves for biocomposites formulated from PBAT and RMB.

the initial biomass; which disappear in the FT-IR results of the RMB, indicating that the lipids present in RMB do not correspond to fatty acids but to other types of lipids such as phospholipids, waxes and sterols, among others, that have been identified in microalgae oil. In addition, fiber and carbohydrates content decreased by 63% in RMB, as polysaccharides and carbohydrates can be partially removed in the biodiesel production process and subsequent RMB neutralization process.

Thermal Analysis of Biocomposites Prepared with PBAT and RMB. A melting process with RMB was used to fabricate biocomposites. The formulations of the biocomposites with 10, 20 and 30% RMB are shown in Table 2.

TGA provides key information about the degradation profile and thermal stability of materials. It is important to know the thermal stability of RMB in order to determine the extrusion conditions and composition of the biocomposites. The DTGA curves also show the effect of the components on thermal properties. On the curves, three states of decomposition can be identified: (i) decomposition of compounds of low molecular weight (26–290 °C), (ii) degradation of carbohydrates and some protein (130–530 °C) and (iii) thermally stable components such as ash, lignin and char (530–775 °C).^{36–38}

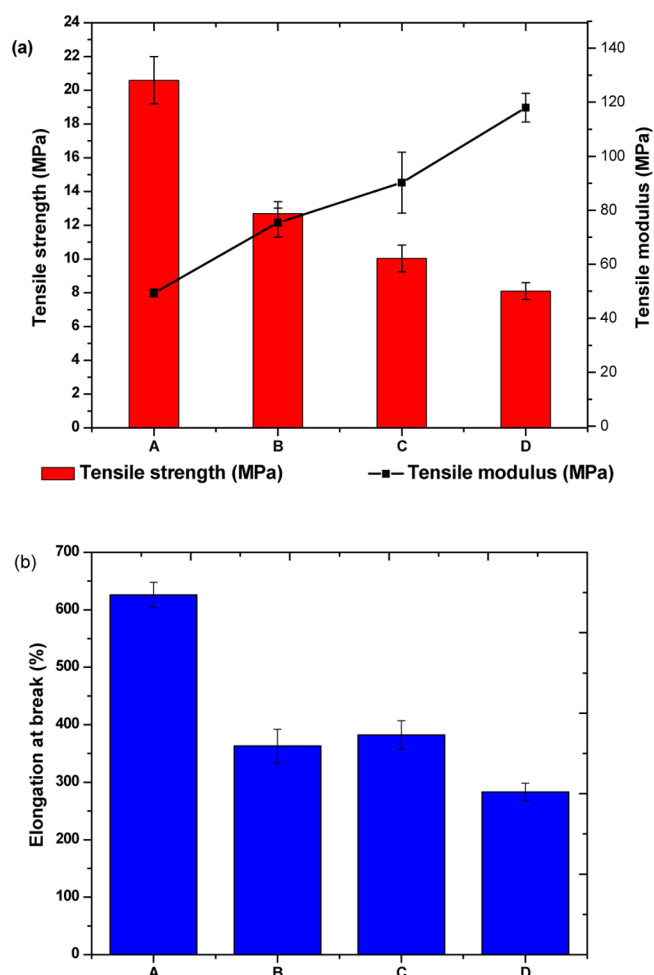


Figure 3. Comparison of the mechanical properties of biocomposites fabricated in this work: (a) tensile strength and tensile modulus and (b) elongation at break. (A) PBAT/RMB (100/0); (B) PBAT/RMB (90/10); (C) PBAT/RMB (80/20); (D) PBAT/RMB (70/30).

The thermal stability of RMB and the PBAT/RMB composites was analyzed using TGA and DTGA experiments. Figure 2a presents the TGA curves for RMB, PBAT and PBAT/RMB composites. The thermal stability of RMB is similar to the results obtained for *Jatropha* meal, but is lower compared to soybean meal and DDGS. According to Diebel et al. (2012),³⁷ the remaining oil content in *Jatropha* meal is 9% and for soybean meal ranges between 0.5 and 2%. This fact corroborates that the remaining lipid content in RMB (7%, see Table 3) reduces its thermal stability. The TGA curve for RMB showed low thermal stability with a T_g (5% mass loss) of 176 °C,³⁷ indicating that RMB extrusion can be only performed using low melting point polymers. On the other hand, PBAT exhibited a decomposition temperature above 350 °C, and this decomposition temperature decreased as the RMB content of the biocomposites increased. DTGA curves for the PBAT/RMB biocomposites showed just one step in the degradation profile, but the maximum peak shifted to lower values when the RMB content of the biocomposites increased. However, the RMB displayed a multistep degradation profile due to its chemical composition (Figure 2b). The remaining lipids in RMB (Table 3) are first degraded, corresponding to the first peak of the DTGA curve, also including the low molecular weight carbohydrates present (7%). The second peak in the

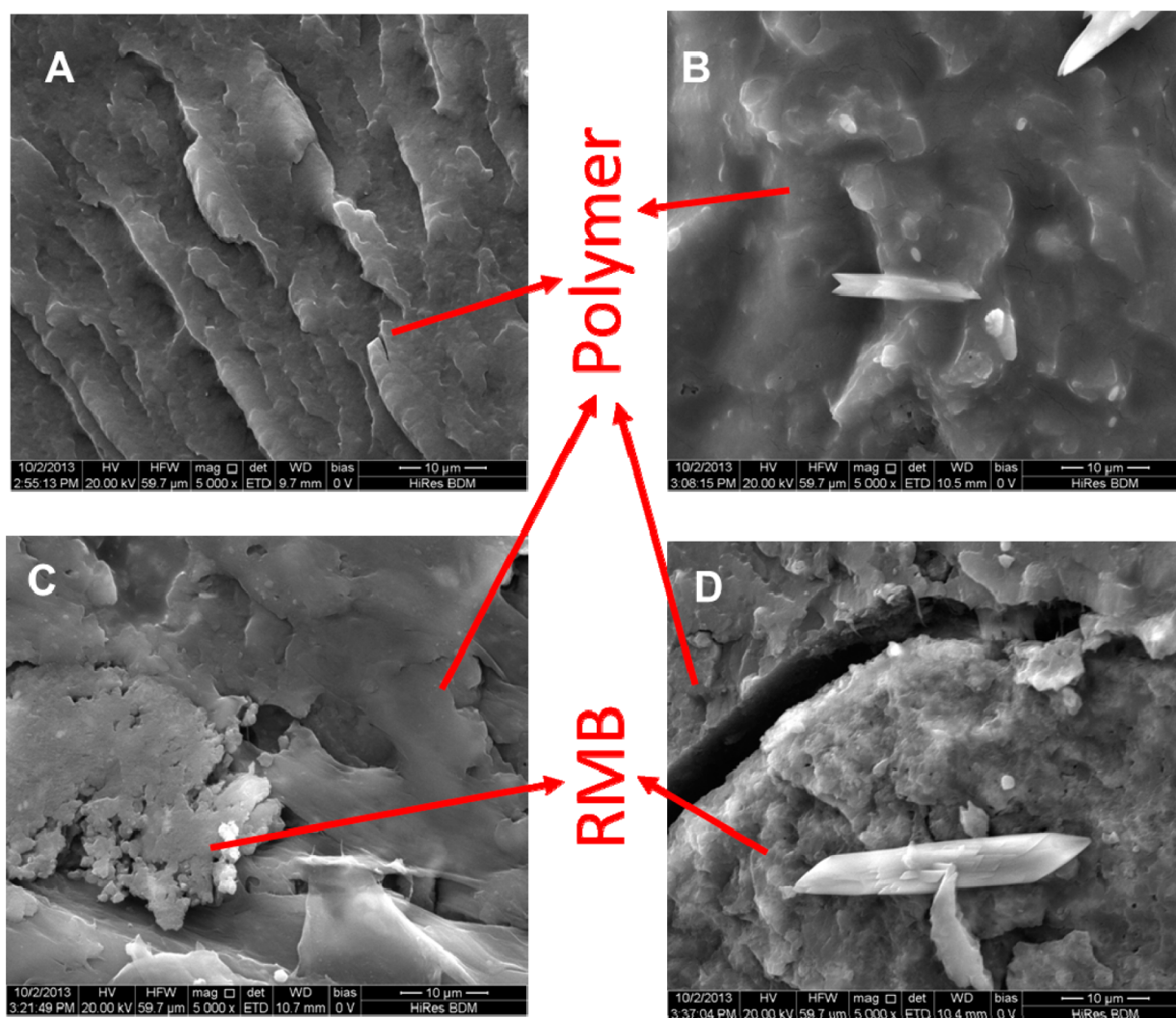


Figure 4. SEM images of cryofractured surface of PBAT/RMB biocomposites fabricated in this work. (A) PBAT/RMB (100/0); (B) PBAT/RMB (90/10); (C) PBAT/RMB (80/20); (D) PBAT/RMB (70/30).

DTGA curve of RMB corresponds to compounds with a higher molecular weight such as polysaccharides and proteins. Finally, the third peak in the third degradation zone corresponds to protein and the high ash content of the biomass associated with the salts present in microalgae cultivation in salt water and nutrients that remain in the RMB. The low fiber content implies low lignin levels, so these components contribute less to the degradation profile of the RMB DTGA curve. Previous publications have shown similar behavior for other additives such as DDGS,³⁶ fiber^{18,19,39} and RMB¹⁵ used in composites.

Characterization of PBAT and PBAT/RMB Composites. Mechanical properties such as tensile strength, tensile modulus and elongation at break of the RMB/PBAT composites containing 10, 20 and 30% of the additive were studied; the results are presented in Figure 3. According to the results, the tensile strength of the biocomposites (PBAT/RMB) was 38, 50 and 61% lower than that of PBAT when the RMB content was increased by 10, 20 and 30%, respectively. Conversely, the tensile modulus increased by 53, 83 and 138%, respectively. Elongation at break of the PBAT/RMB biocomposites with 10, 20 and 30% RMB fell by 60, 25 and 45%, respectively. The flexural properties, flexural strength and flexural modulus, increased with RMB content (Table 2). Therefore, when the

RMB content increased, the tensile modulus and flexural modulus improved, but elongation at break and tensile strength decreased. Impact strength did not change at 10, 20 RMB (Table 2), but RMB improved the impact strength of the biocomposites at 30%. Most authors have explained the decrease in mechanical properties of composites as the result of poor interaction of the additive and the polymer matrix. Incorporating RMB into PBAT produces biocomposites with inferior mechanical properties. For natural fiber composites, the mechanical properties depend on the nature of the additive, the polymer matrix and the fibers. Incorporating natural fibers reduces mechanical properties, but increases resistance to impact in most cases. This effect is not observed for RMB, up to an incorporation of 30% RMB into the composites. Protein-rich materials like RMB have poor mechanical properties because proteins engage in intermolecular interactions that cause them to form complex structures by binding together to form microfibrils, reducing the polymer matrix-additive interaction. To obtain evidence of the morphology of RMB and its dispersion in the polymer matrix, SEM studies were performed.

Density is another important property for determining the applications of composites, as generally the aim is to obtain

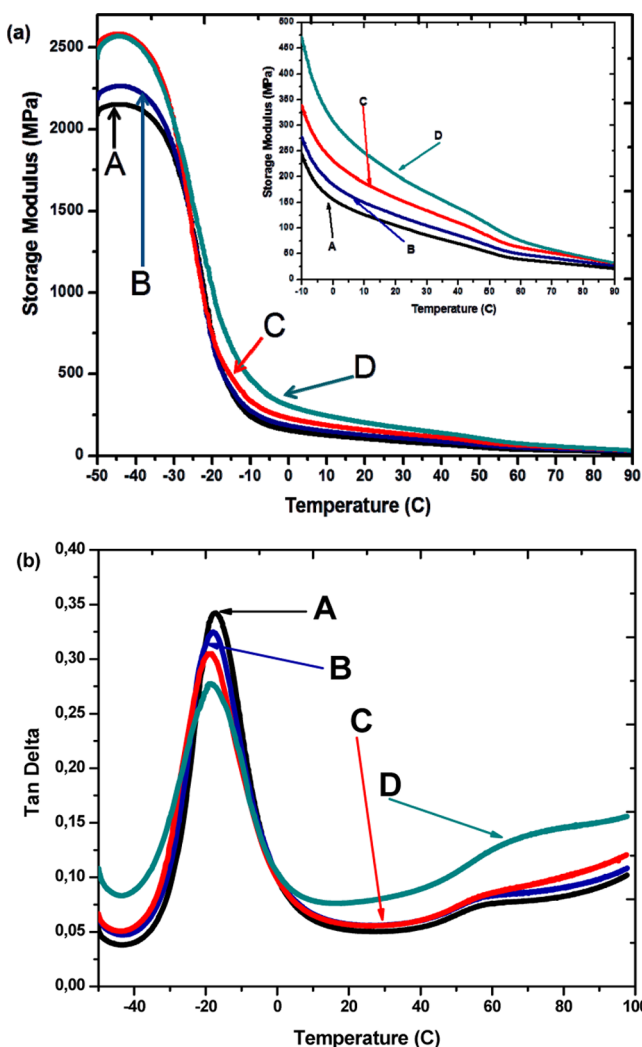


Figure 5. Dynamic mechanical properties of neat PBAT and PBAT/RMB composites: (A) PBAT/RMB (100/0), (B) PBAT/RMB (90/10), (C) PBAT/RMB (80/20) and (D) PBAT/RMB (70/30).

lighter materials. Of the most frequently used materials in composites like fiber and fiberglass, natural fibers are considered to have a lower density ($1.3\text{--}1.5\text{ g/cm}^3$) compared to fiberglass (2.6 g/cm^3). Nagarajan et al. (2013)¹⁸ compared the density of different composites manufactured with 30% natural fibers such as miscanthus, soy stalk, and switchgrass, among others. Incorporating a light material into the polymer matrix causes the density of the final composite to change slightly.

The density of byproducts rich in proteins such as soybean meal (40–55%) and Jatropha meal (60%) is low compared to the density of natural fibers. For example, soybean meal has a density of $0.727 \pm 0.020\text{ (g/cm}^3\text{)}$ and Jatropha meal has a density of $0.668 \pm 0.016\text{ (g/cm}^3\text{)}$. In this research, the density of RMB as a byproduct of biodiesel production has been determined to be $0.8409 \pm 0.010\text{ (g/cm}^3\text{)}$. Table 2 shows the density obtained for composites with 10, 20 and 30% RMB compared to the PBAT polymer matrix. Although a slight decrease in the density of the fabricated composites was expected, a similar behavior to composites fabricated with natural fibers was observed, as the density of composites with RMB slightly increased to reach $1.28\text{ (g/cm}^3\text{)}$ for PBAT/RMB (70/30) compared to PBAT/RMB (100/0). This behavior may

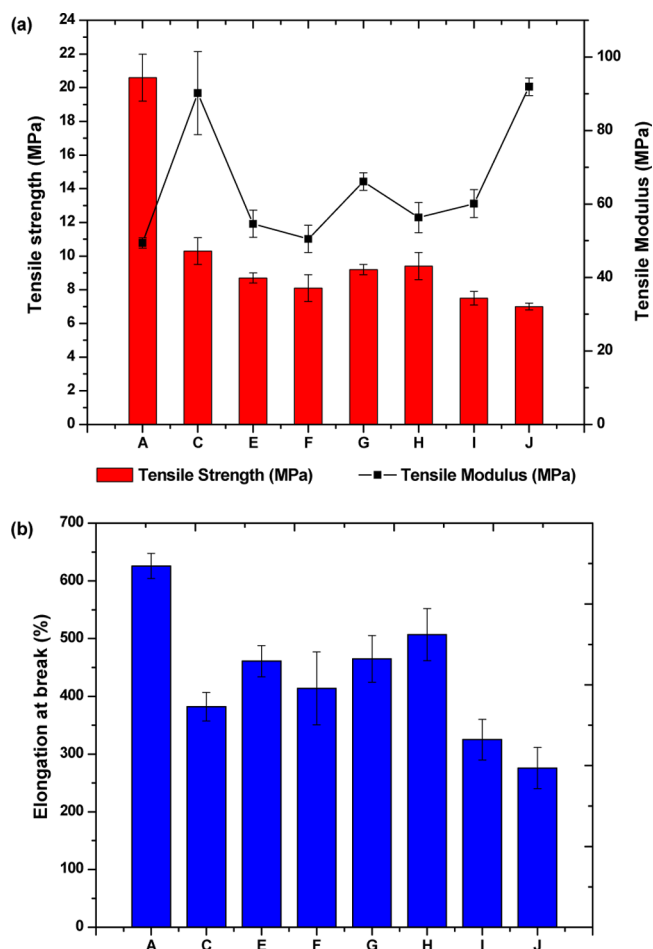


Figure 6. Comparison of the effect of plasticizer use on mechanical properties of biocomposites fabricated in this work: (a) tensile strength and tensile modulus and (b) elongation at break. (A) PBAT/RMB (100/0); (C) PBAT/RMB (80/20); (E–J) PBAT/RMB composites with different compatibilization treatments (see Table 4).

be related to the small amounts of RMB used and the possible formation of a denser matrix.

Table 2 provides the HDT of the biocomposites. HDT is also an important property because it enables to determine the maximum temperature at which the material can be used as rigid material. HDT is defined as the temperature at which a material deflects 0.25 mm under a load of 0.455 or 1.82 MPa. The HDT value for PBAT is low compared to other biodegradable polymers. It is evident from Table 2 that the HDT value does not vary in composites fabricated with RMB. Because RMB is a material rich in proteins, it offers low resistance compared to natural fibers, which provide additional reinforcement to composites, thereby increasing HDT values.

The SEM images of cryofractured surface (Figure 4) can explain why the mechanical properties of the matrix decreased when RMB is added. Figure 4A,B,C,D shows the SEM images of the polymeric matrix of PBAT/RMB with 0, 10, 20 and 30% RMB, respectively. RMB can be processed with PBAT under the conditions used in this study, reducing the mechanical properties of the polymer matrix. It was observed that when the filler was added to the biocomposite the polymeric matrix changed, generating gaps and agglomerations of RMB. Figure 4A shows the polymer morphology and Figure 4B shows how it changes when 10% RMB is incorporated. The greatest

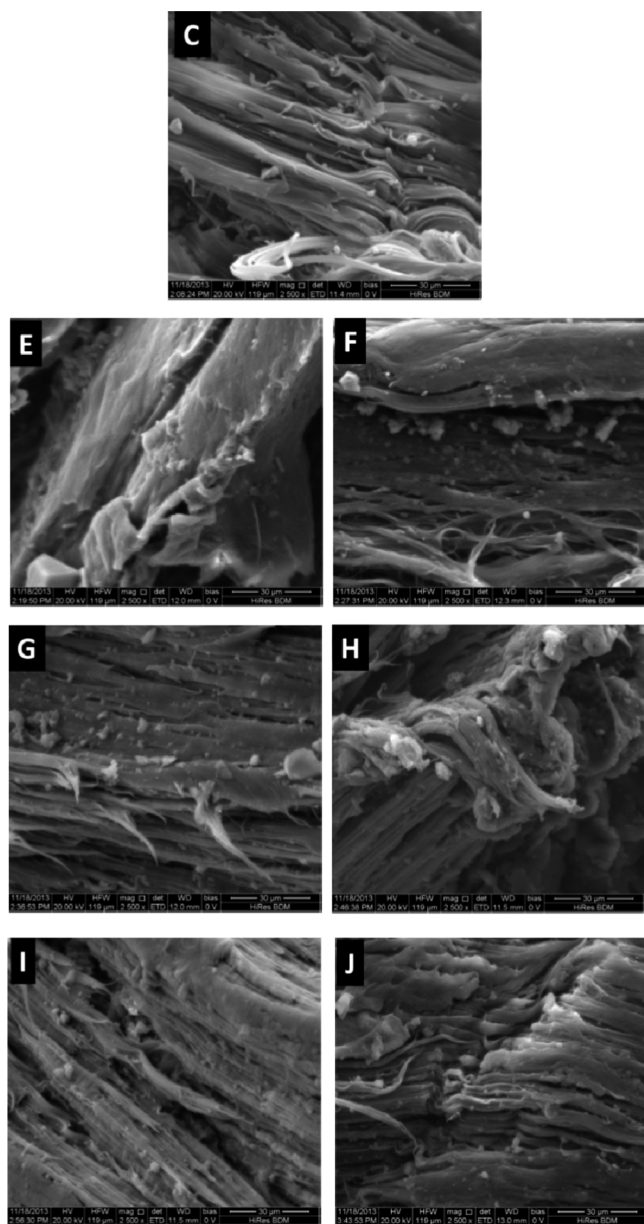


Figure 7. SEM images of biocomposites fabricated in this work. Comparison of (A) PBAT/RMB (100/0), (C) PBAT/RMB (80/20) and (E–J) PBAT/RMB composites with different compatibilization treatments (see Table 4).

differences are observed, however, for composites fabricated with 20 and 30% RMB (Figure 4C,D). RMB particles of different sizes can be observed in the matrix but they are not completely incorporated into it. The effect is more apparent when 30% RMB is incorporated. The fabricated composite shows a clear separation between the polymer matrix and RMB phase, which would explain the poor mechanical properties. Similar effects have been seen in previous studies. Hassan et al. (2008)⁴⁰ observed that certain properties of polyethylene were affected when seaweed powder was incorporated as filler. Similar results were obtained by Gravier et al. (2004)⁴¹ using soybean concentrate with Eastar Bio Copolyester, where the particles of soybean concentrate formed poorly distributed agglomerations on the matrix, reducing the properties of the polymer.

Finally, the PBAT/RMB composites were analyzed using dynamic mechanical techniques. As shown in Figure 5, increasing the RMB content (10, 20 and 30%) in biocomposites increased the storage modulus value. This can best be observed in the graph in Figure 5a, which demonstrates that upon addition of RMB the rigidity of the polymer matrix improved, increasing its strength. However, the δ peak decreased when RMB content increased (Figure 5b). The maximum peak also shifted to lower temperature values. This was due to the incorporation of the additive into the polymer matrix, which restricted the mobility of the polymer chains. Thus, it was necessary to improve the RMB's compatibility with the polymer matrix.

Characterization of PBAT/RMB Plasticized Biocomposites. In this study, glycerol and urea were used as plasticizers to improve the mechanical properties of PBAT/RMB biocomposites (Tables 1 and 2 show the RMB pretreatment with plasticizers and the respective formulations used in the biocomposites). Figure 6 shows the tensile strength and tensile modulus values of the biocomposites with RMB preformulations, showing the effect of using glycerol as a plasticizer in the presence and absence of water (samples E, F and G). Samples H, I and J show the comparison between using glycerol as a plasticizer and increasing the percentage of urea (denaturant). It can be seen that the plasticizing process improved in the presence of water (sample G). Sample G is considered the best formulation as it improved the elongation at break of biocomposites. This formulation was used to analyze the effect of urea content as a pretreatment for RMB. Samples H, I and J show the results of using glycerol as a plasticizer and increasing the percentage of urea. In terms of improved mechanical properties of the composites, the tensile strength did not change with plasticization compared with PBAT/RMB (80/20). The tensile modulus for samples E, F and H decreased 11, 2 and 14% compared with PBAT, and for PBAT/RMB (80/20) without plasticization (sample C), it decreased 83%. Finally, elongation at break decreased 39% for the composite PBAT/RMB (80/20) without plasticization (sample C); however, for the plasticized sample H, the decrease was only 19% compared to PBAT. The best result was obtained for RMB with 30% glycerol and 7.5 phr of urea in the presence of water. When the urea content increased in the RMB pretreatment, the mechanical properties declined compared to PBAT/RMB (80/20). Chen and Zhang (2010) obtained good results using glycerol as a plasticizer for soybean protein concentrate (SPC) in the formulation of SPC/PBAT composites⁴² whereas Tunmala et al. (2006) used a similar procedure for plasticizing soybean flour.⁴³ In this case, considering that RMB contains 40% protein, employing plasticizers used for SPC (with 52% protein) and soybean concentrate (with 65% protein) yielded good results in terms of matrix–filler interaction.

Figure 7 shows the SEM images of all the biocomposites with plasticized RMB. It can be seen in the images that RMB was dispersed in all the samples with plasticized RMB, compared to PBAT/RMB (80/20). Figure 8 shows the comparison of the biocomposites with 0, 20 RMB without pretreatment and biocomposites H. Composite H was the best formulation of pretreated RMB. The SEM images show that untreated RMB agglomerated in the matrix and formed large particles, but when plasticized RMB was used, it dispersed in the polymer. It is evident that biocomposite H (plasticized RMB with 20% glycerol and 7.5 phr of urea) had the highest dispersion on the polymer matrix. This was further confirmed by the confocal

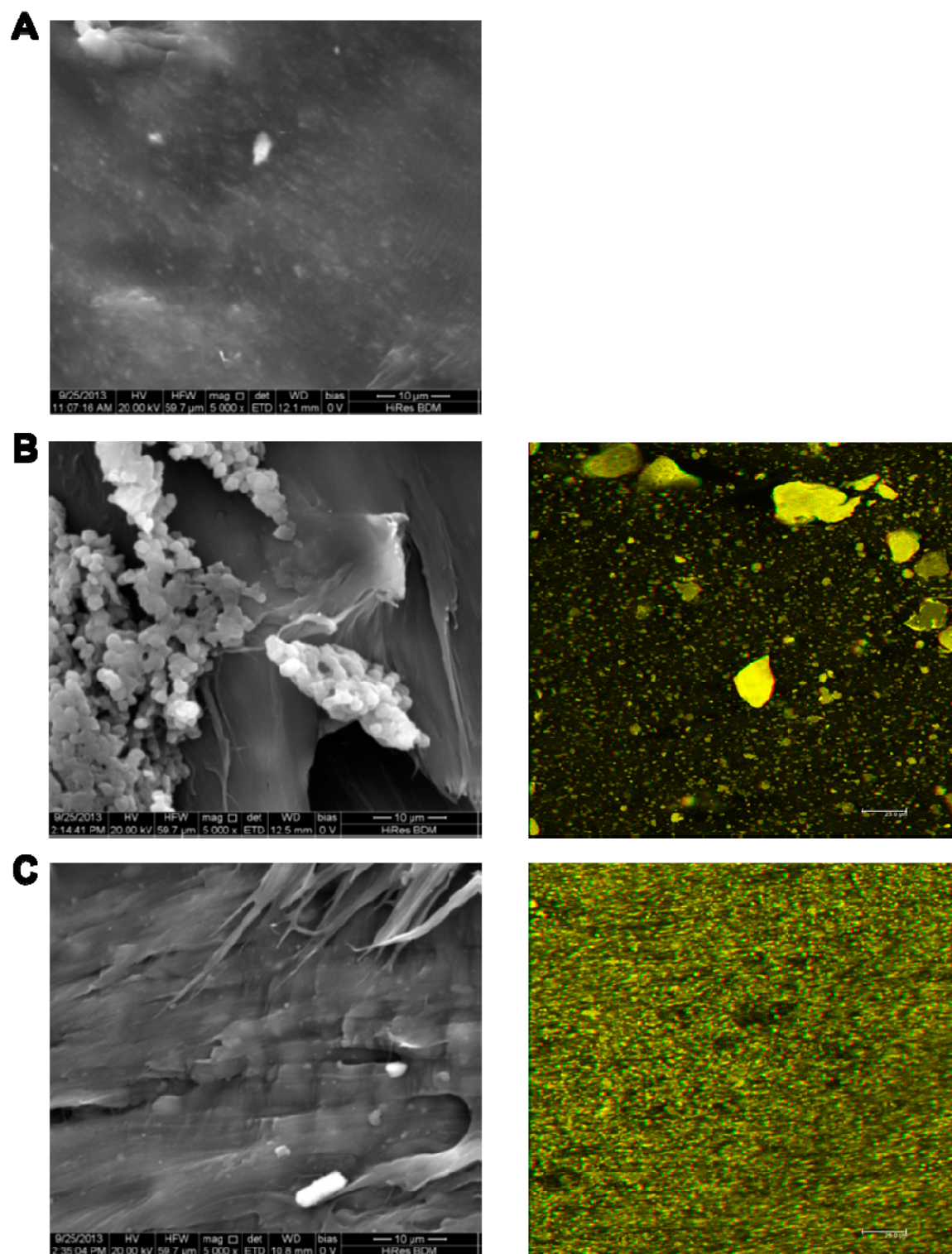


Figure 8. SEM image of transverse cutting (left) and CLSM (right) images of biocomposites fabricated in this work. Comparison of (A) PBAT/RMB100/0) and (B) PBAT/RMB (80/20) with (C) PBAT/RMB composites with plasticization treatment sample H (see Table 4).

images, which clearly show the difference in the dispersion of RMB. Plasticizers such as glycerol are used to modify the structure of the proteins, which form complex three-dimensional structures due to molecular interactions. Glycerol and urea have been used as plasticizers to promote interaction between protein groups, interrupting the intramolecular interactions and enabling the protein structure to interact with the polymer matrix.⁴⁰ With the high content of protein in

RMB, it is apparent that this procedure generates interesting results in terms of interaction of PBAT and RMB. The storage modulus and $\tan \delta$ results (see Figure 9) show that when the matrix is compatibilized with RMB, there is a higher mobility of polymer chains and the curves of all the composites with plasticized RMB are very similar to the curve for PBAT. This corroborates the plasticization and denaturation effect of

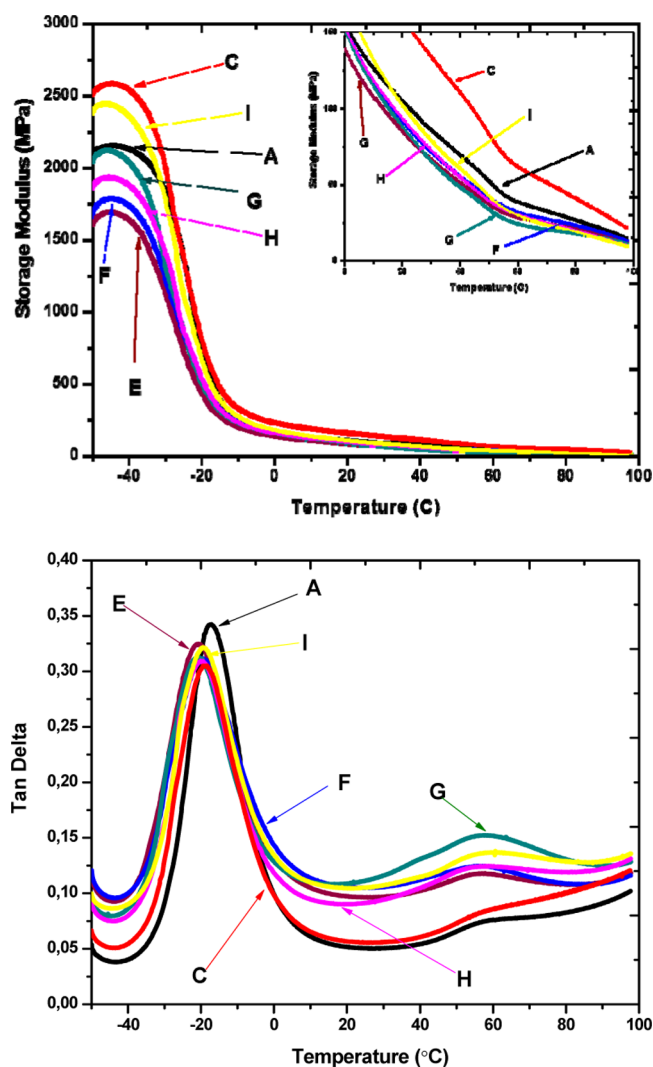


Figure 9. Dynamic mechanical properties of neat PBAT and PBAT/RMB composites with plasticization treatments (see Table 4).

glycerol and urea, which produced greater mobility of the RMB microfibrils in the polymer matrix (see Figure 9).

CONCLUSIONS

The chemical composition of RMB, made up of protein and carbohydrates, generates its low thermal stability, enabling its use only with polymers that also present low melting points. A series of RMB and PBAT biocomposites with and without plasticizer were prepared by extrusion and injection molding. The studies show that RMB can be used to fabricate biocomposites with PBAT, with the best results occurring with extrusion of 20% RMB. In terms of improved mechanical properties of the composites, plasticization was only able to improve tensile modulus and elongation when comparing the results with composite PBAT/RMB (80/20); the best formulation and optimal plasticization was achieved with 20% glycerol and 7.5 phr of urea. Using PBAT, a biodegradable polymer, and RMB to fabricate biocomposites can reduce their overall cost. These types of materials can be used for agricultural films that degrade over time once they come into contact with soil. This study also provides new data on a sustainable way to produce biodiesel from microalgae, generating new related coproducts.

AUTHOR INFORMATION

Corresponding Author

*S. Torres. E-mail: storresg@desertbioenergy.cl. Phone +56 45 2734037.

Author Contributions

All authors have given approval to the final version of the paper.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work received financial support from the FONDECYT Chile Grant 11121591; "Insertion of Advanced Human Capital in Chile's Productive Sector," (Project No. 78110106); Desert Bioenergy S.A. (from Innova-CORFO); the Ontario Ministry of Economic Development and Innovation (MEDI), Ontario Research Fund—Research Excellence Round 4 program; the Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA)—University of Guelph Bioeconomy-Industrial uses Research Program; OMAFRA—New Directions & Alternative Renewable Fuels Research program; the Natural Sciences and Engineering Research Council (NSERC), Canada for the Discovery grants individual (to Mohanty).

ABBREVIATIONS

CLSM = confocal laser scanning microscopy
 DDGS = dried distiller's grains with solubles
 DGGs = distiller's dried grains with solubles
 FT-IR = Fourier transform infrared spectroscopy
 PBAT = poly(butylene adipate-co-terephthalate)
 PBS = poly(butylene succinate)
 PP = polypropylene
 RMB = residual microalgae biomass
 TGA = thermogravimetric analysis
 SEM = scanning electron microscopy

REFERENCES

- (1) Ahmad, A. L.; Yasin, N. H. M.; Derek, C. J. C.; Lim, J. K. Microalgae as a sustainable energy source for biodiesel production: A review. *Renew. Sust. Energy Rev.* **2011**, *15* (1), 584–593.
- (2) Issariyakul, T.; Dalai, A. K. Biodiesel from vegetable oils. *Renewable Sustainable Energy Rev.* **2014**, *31*, 446–471.
- (3) Atabani, A. E.; Silitonga, A. S.; Anjum, I.; Mahlia, T. M. I.; Masjuki, H. H.; Mekhilef, S. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable Sustainable Energy Rev.* **2012**, *16* (4), 2070–2093.
- (4) Wang, Y.; Ou, S.; Liu, P.; Xue, F.; Tang, S. Comparison of two different processes to synthesize biodiesel by waste cooking oil. *J. Mol. Catal. A: Chem.* **2006**, *252* (1–2), 107–112.
- (5) Uzun, B. B.; Kılıç, M.; Özbay, N.; Pütün, A. E.; Pütün, E. Biodiesel production from waste frying oils: Optimization of reaction parameters and determination of fuel properties. *Energy* **2012**, *44*, 347–351.
- (6) Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306.
- (7) González-Fernández, C.; Bernet, N.; Steyer, J. P. Impact of microalgae characteristics on their conversion to biofuel. Part I: Focus on cultivation and biofuel production. *Biofuels, Bioprod. Bioref.* **2012**, *6*, 105–113.
- (8) Ashokkumar, V.; Rengasamy, R. Mass culture of *Botryococcus braunii* Kutz. under open raceway pond for biofuel production. *Bioresour. Technol.* **2012**, *104*, 394–399.
- (9) Delrue, F.; Setier, P.; Sahut, C.; Cournac, L.; Roubaud, A.; Peltier, G.; Froment, A.-K. An economic, sustainability, and energetic model of

biodiesel production from microalgae. *Bioresour. Technol.* **2012**, *111*, 191–200.

(10) Halim, R.; Danquah, M. K.; Webley, P. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnol. Adv.* **2012**, *30* (3), 709–732.

(11) Miao, X.; Wu, Q. Biodiesel production from heterotrophic microalgal oil. *Bioresour. Technol.* **2006**, *97* (6), 841–846.

(12) Chen, L.; Liu, T.; Zhang, W.; Chen, X.; Wang, J. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. *Bioresour. Technol.* **2012**, *111*, 208–214.

(13) Hidalgo, P.; Toro, C.; Ciudad, C.; Navia, R. Advances in direct transesterification of microalgal biomass for biodiesel production. *Rev. Environ. Sci. Biotechnol.* **2012**, *12*, 179–199.

(14) Hidalgo, P.; Toro, C.; Ciudad, G.; Schober, S.; Mittelbach, M.; Navia, R. Evaluation of different operational strategies for biodiesel production by direct transesterification of microalgal biomass. *Energy Fuels* **2014**, *28*, 3814–3820.

(15) Toro, C.; Reddy, M. M.; Navia, R.; Rivas, M.; Misra, M.; Mohanty, A. K. Characterization and application in biocomposites of residual microalgal biomass generated in third generation biodiesel. *J. Polym. Environ.* **2013**, *21*, 944–951.

(16) Jiang, L.; Chen, F.; Qian, J.; Huang, J.; Wolcott, M.; Liu, L.; Zhang, J. Reinforcing and toughening effects of bamboo pulp fiber on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) fiber composites. *Ind. Eng. Chem. Res.* **2010**, *49* (2), 572–577.

(17) Bledzki, A. K.; Jaszkiwicz, A. Mechanical performance of biocomposites based on PLA and PHBV reinforced with natural fibres – A comparative study to PP. *Compos. Sci. Technol.* **2010**, *70* (12), 1687–1696.

(18) Nagarajan, V.; Mohanty, A.; Misra, M. Sustainable green composites: Value addition to agricultural residues and perennial grasses. *ACS Sustainable Chem. Eng.* **2013**, *1*, 325–333.

(19) Zhang, K.; Misra, M.; Mohanty, A. K. Toughened sustainable green composites from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) based ternary blends and *Miscanthus* biofiber. *ACS Sustainable Chem. Eng.* **2014**, *2*, 2345–2354.

(20) Wu, C. S. Utilization of peanut husks as a filler in aliphatic–aromatic polyesters: Preparation, characterization, and biodegradability. *Polym. Degrad. Stab.* **2012**, *97* (11), 2388–2395.

(21) Raquez, J. M.; Nabar, Y.; Narayan, R.; Dubois, P. Novel high-performance talc/poly[(butylene adipate)-co-terephthalate] hybrid materials. *Macromol. Mater. Eng.* **2008**, *293* (4), 310–320.

(22) Sousa, G. M.; Soares Júnior, M. S.; Yamashita, F. Active biodegradable films produced with blends of rice flour and poly(butylene adipate co-terephthalate): Effect of potassium sorbate on film characteristics. *Mater. Sci. Eng. C* **2013**, *33*, 3153–3159.

(23) Bengtsson, M.; Gatenholm, P.; Oksman, K. The effect of crosslinking on the properties of polyethylene/wood flour composites. *Compos. Sci. Technol.* **2005**, *65* (10), 1468–1479.

(24) Paunikallio, T.; Suvanto, M.; Pakkanen, T. T. Grafting of 3-(trimethoxysilyl)propyl methacrylate onto polypropylene and use as a coupling agent in viscose fiber/polypropylene composites. *React. Funct. Polym.* **2008**, *68* (3), 797–808.

(25) Rachini, A.; Mougin, G.; Delalande, S.; Charneau, J. Y.; Barrès, C.; Fleury, E. Hemp fibers/polypropylene composites by reactive compounding: Improvement of physical properties promoted by selective coupling chemistry. *Polym. Degrad. Stab.* **2012**, *97* (10), 1988–1995.

(26) Sun, S.; Song, Y.; Zheng, Q. Morphologies and properties of thermo-molded biodegradable plastics based on glycerol-plasticized wheat gluten. *Food Hydrocolloids* **2007**, *21* (7), 1005–1013.

(27) Liu, B.; Jiang, L.; Liu, H.; Zhang, J. W. Synergetic effect of dual compatibilizers on in situ formed poly(lactic acid)/soy protein composites. *Ind. Eng. Chem. Res.* **2010**, *49*, 6399–6406.

(28) Nabar, Y.; Raquez, J. M.; Dubois, P.; Narayan, R. Production of starch foams by twin-screw extrusion: Effect of maleated poly(butylene adipate-co-terephthalate) as a compatibilizer. *Biomacromolecules* **2005**, *6* (2), 807–817.

(29) Al-Itry, R.; Lamnawar, K.; Maazouz, A. Improvement of thermal stability, rheological and mechanical properties of PLA, PBAT and their blends by reactive extrusion with functionalized epoxy. *Polym. Degrad. Stab.* **2012**, *97*, 1898–1914.

(30) Jang, Y. H.; Han, S. O.; Sim, I. N.; Kim, H. I. Pretreatment effects of seaweed on the thermal and mechanical properties of seaweed/polypropylene biocomposites. *Composites, Part A* **2013**, *47*, 83–90.

(31) Torres, S.; Navia, R.; Campbell Murdy, R.; Cooke, P.; Misra, M.; Mohanty, A. Green composites from microalgae biomass and bioplastic: Processing and compatibilization. *30th International Conference of The Polymer Processing Society*; Cleveland, OH, June 8–12, 2014; Polymer Processing Society: Lawrence, KS, 2014.

(32) Hernandez- Izquierdo, V. M.; Krochta, J. M. Thermoplastic processing of proteins for film formation - A Review. *J. Food Sci.* **2008**, *73*, 30–39.

(33) Zhou, X.; Mohanty, A.; Misra, M. A new biodegradable injection moulded bioplastic from modified soy meal and poly(butylene adipate-co-terephthalate): Effect of plasticizer and denaturant. *J. Polym. Environ.* **2013**, *21*, 615–622.

(34) Kijchavengkul, T.; Auras, R.; Rubino, M. Measuring gel content of aromatic polyesters using FTIR spectrophotometry and DSC. *Polym. Test.* **2008**, *27* (1), 55–60.

(35) Olivato, J. B.; Grossmann, M. V. E.; Yamashita, F.; Eiras, D.; Pessan, L. A. Citric acid and maleic anhydride as compatibilizers in starch/poly(butylene adipate-co-terephthalate) blends by one-step reactive extrusion. *Carbohydr. Polym.* **2012**, *87* (4), 2614–2618.

(36) Zarrinbakhsh, N.; Misra, M.; Mohanty, A. K. Biodegradable green composites from distiller's dried grains with solubles (DDGS) and a polyhydroxy(butyrate-co-valerate) (PHBV)-based Bioplastic. *Macromol. Mater. Eng.* **2011**, *296* (11), 1035–1045.

(37) Diebel, W.; Reddy, M. M.; Misra, M.; Mohanty, A. K. Material property characterization of co-products from biofuel industries: Potential uses in value-added biocomposites. *Biomass Bioenergy* **2012**, *37*, 88–96.

(38) Muniyasamy, S.; Reddy, M. M.; Misra, M.; Mohanty, A. K. Biodegradable green composites from bioethanol co-product and poly(butylene adipate-co-terephthalate). *Ind. Crops Prod.* **2013**, *43*, 812–819.

(39) Nagarajan, V.; Misra, M.; Mohanty, A. K. New engineered biocomposites from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/poly(butylene adipate-co-terephthalate) (PBAT) blends and switchgrass: Fabrication and performance evaluation. *Ind. Crop. Prod.* **2013**, *42*, 461–468.

(40) Hassan, M. M.; Mueller, M.; Wagners, M. H. Exploratory study on seaweed as novel filler in polypropylene composite. *J. Appl. Polym. Sci.* **2008**, *109*, 1242–1247.

(41) Graiver, D.; Waikul, L. H.; Berger, C.; Narayan, R. Biodegradable soy protein-polyester blends by reactive extrusion process. *J. Appl. Polym. Sci.* **2004**, *92* (5), 3231–3239.

(42) Chen, F.; Zhang, J. W. Effects of plasticization and shear stress on phase structure development and properties of soy protein blends. *ACS Appl. Mater. Interfaces* **2010**, *2* (11), 3324–3332.

(43) Tunmala, P.; Liu, W.; Lawrence, T. D.; Mohanty, A. K.; Manju, M. Influence of plasticizers on thermal and mechanical properties and morphology of soy-based bioplastics. *Ind. Eng. Chem. Res.* **2006**, *45*, 7491–7496.