
Research article

Exploration of cassava clones for the development of biocomposite films

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Abstract: Due to the growing interest in developing bioplastic films from renewable sources, the performance of biocomposite films produced of native starch from cassava clones reinforced with cassava bagasse was explored. The biocomposites were prepared from the starch of cassava clones MMEXV5, MMEXV40, and MMEXCH23, reinforced with bagasse at 1%, 5%, and 15%. Their structural, mechanical, and thermal properties were subsequently assessed. When analyzing the starch, differences in the intensities of the Raman spectra exhibit a possible variation in the amylose-amylopectin ratio. In the biocomposites, the bagasse was efficiently incorporated into polymeric matrixes and their thermogravimetric analysis revealed the compatibility of the matrix-reinforcement. The starch films from the MMEXV40 clone showed better tension (2.53 MPa) and elastic modulus (60.49 MPa). The assessed mechanical properties were also affected by bagasse concentration. Because of the above, the MMEXV40 cassava clone showed potential to develop

polymeric materials, given its tuberous roots high yield, starch extraction, and good performance in its mechanical properties. At the same time, the starch source (clone) and the bagasse concentration interfere with the final properties of the biocomposites.

Keywords: *Manihot esculenta* Crantz clones; amylose–amylopectin; biocomposites; thermoplastic starch; bagasse

1. Introduction

Plastics from fossil origin are indispensable materials for various human activities [1]; however, they relate to waste accumulation in the environment, damage to aquatic and terrestrial fauna, and effects on human health [2–5]. Nowadays, 8.3 billion tons of plastic have been manufactured globally; however, if production and consumption trends continue in the next three decades, up to 12 billion tons could end up in open-air dumps or the environment [6], especially by industrial packaging products [7]. Therefore, an appropriate waste management plan, certain government regulations, and using innovative solutions are necessary to decrease the inconveniences of conventional plastics [8].

Bioplastics are materials proposed as an eco-friendly alternative, where starch is considered one of the most widely used biopolymers [7], due to its abundance, and renewable and economic nature [9,10]. In this regard, corn is a raw material to extract polysaccharides for bioplastics manufacture [11]. It is one of the most important cereals in the world, cultivated on 192 million hectares, with an average yield of 5.6 t ha⁻¹ [12]. Nevertheless, for bioplastics production, it is necessary to explore other resources not competing with food production, given the continuous increase in the demand for food products because of population growth [13]. It should be noted that cassava (*Manihot esculenta* Crantz) is a tropical tuber with a world average yield of 11.26 t ha⁻¹ [12], which stands out from other starchy crops for its ability to produce in infertile soils, under water stress, and for its drought adaptability [14–16]. Moreover, it can produce ~74% to 90% starch (dry base [17,18]) which is easy to extract [19]. This, together with the clarity of its paste and gel stability, including the low gelatinization temperature of its starch, makes it an ideal raw material for the sustainable development of biodegradable materials [20].

It is worth mentioning that starch-based bioplastic films have low mechanical and barrier properties [21,22], due to the hydrophilic nature of their polymer [23], which is why thermoplastic starch conversion (TPS) or using mixtures with other polymers is frequent [24]. Nevertheless, the origin, morphological and structural properties, and chemical composition of native starch are factors that can induce a wide range of properties in their films [21–26]. Also, incorporating natural fibers into polymeric matrixes improves the functional properties of bioplastic films [27], which have attracted attention because of their bioavailability, renewability, high resistance, rigidity, low density, and cost [28,29].

To this regard, the cassava industry produces a large amount of lignocellulosic solid waste [30], which could be used as reinforcing agents in biocomposites. It should be noted that up to 2.5 tons of bagasse are generated for each extracted starch ton [31], and from 100 to 200 kg of peels per ton of processed roots [32]. Although both byproducts can be used as reinforcement materials in biocomposites, bagasse has greater efficiency, partly due to its high residual starch content [33]. Thus,

its incorporation into polymeric matrixes can contribute to sustainable waste management and offer added value to their byproducts [34].

In Mexico, cassava crop is done following traditional agricultural systems for self-consumption [35,36]. Despite this, a 12.58 t ha^{-1} yield is reached, which is 11.72% higher than the world average [12]. At the same time, there is great native germplasm diversity with contrasting morpho-agronomic and industrial characteristics [37–40], not yet extensively explored for biodegradable films development. Furthermore, the importance in Mexico of cassava to produce bioplastics is that it is an alternative to the usage of starch from food sources such as corn, a staple food cereal, which requires to import up to 17 million tons per year to meet its demand [41,42]. Therefore, the objective of this research was to explore the structural, mechanical, and thermal properties of biocomposite films made with starches from three native cassava clones, MMEXV5, MMEXV40, and MMEXCH23, reinforced with three bagasse concentrations (1%, 5%, and 15%) as reinforcement.

2. Materials and methods

2.1. Raw material obtaintion

The cassava clones, MMEXV5, MMEXV40, and MMEXCH23, were sown and harvested 10 months after sowing (February–December 2018 agricultural cycle) at the Cotaxtla Experimental Field ($18^{\circ}56'11.28'' \text{ NL}$ and $96^{\circ}11'49.53'' \text{ WL}$) of the National Institute of Forestry, Agricultural, and Livestock Research (NIFALR), Veracruz, Mexico. Later, in the General Uses Laboratory of the Faculty of Biological and Agricultural Sciences, from the University of Veracruz, the native starch was extracted from the tuberous roots, following the methodology by López et al. [43], and Vargas et al. [44], with slight modifications. To do this, the roots were immersed in a NaClO solution (250 ppm L^{-1}) for 10 min, then peeled. After this, the pulp was removed and cut into small pieces, then grounded in a juice extractor (Haus, model: 74.20304, China) until a whitish suspension was obtained and the bagasse byproduct separated. The suspension was filtered through a mesh plastic (0.5 mm); 500 mL distilled water was added to the filtrate, and then stirred, the starch was let to settle for three hours. Afterward, it was decanted until a starch paste (settled starch) was obtained. This procedure was repeated three times. The fresh bagasse was squeezed in a plastic mesh until a starch suspension was obtained. The resulting settled starch was then placed in an oven (Ecoshel 9023A, United States) at a 50°C temperature for 24 h, while the fresh bagasse was dried at 50°C for 72 h [34].

2.2. Native starch and bagasse characterization

The diameter of the native starch granules was determined with optical microscope images (Leica DMLM, Germany), for which 120 granules were measured with the Image Pro-Plus software. Also, the amylose-amylopectin ratio was qualitatively calculated by comparing the average of three Raman spectra per starch (DXR Raman Microscope, Thermo Scientific, United States). For this, a small starch sample was placed in an aluminum sample holder. Samples were subjected to a 100 mW power laser for excitation and scattered radiation was collected at 180° . For each spectrum, an average of 1024 scans were taken at a 4 cm^{-1} resolution. It should be noted that Raman spectroscopy has been proposed to evaluate various organic compounds content in samples [45–47].

Meanwhile, the distribution of the dry bagasse particles of the three cassava clones was assessed by a set of sieves with mesh sizes of 250 and 75 μm (Mont-Inox, MON200A060 and MON200A200, Mexico). The sieving procedure was carried out manually for 5 min. The retained fractions on each mesh were separated and weighed on an analytical balance (Denver Instrument A-200DS, United States). Due to a higher proportion of bagasse in the roots of the MMEXCH23 clone reported from previous research [48], and the reinforcement efficiency of <300 μm particle sizes [49,50], the cassava bagasse of the MMEXCH23 clone with a 250–75 μm particle size was used as a reinforcing agent for the polymeric matrixes. The microscopic morphologies of the samples were observed by scanning electron microscope (JSM 7600F, JEOL, Japan) using 1 kV acceleration voltage and a thin graphite layer as a conductive adhesive to fix the sample and to avoid images variation.

2.3. Biocomposite films

2.3.1. Preparation

Biocomposite films were prepared using a casting method. The film-forming solution included 5 g of cassava starch dispersed with 100 mL distilled water, glycerol as a plasticizer (0.39 g dry starch), and the addition of 1%, 5%, and 15% of dry bagasse by weight of starch. The bagasse was pre-mixed with the starch to achieve good particle dispersion [51]. The suspensions were gelatinized at 90 °C for 20 min under vigorous mechanical shaking (Thermo Scientific Cimarrec, United States). The film-forming solution was poured into a 12 × 13 cm cellulose acetate container. To facilitate the films' removal, the container was previously impregnated with a light vegetable oil layer (Pam Spray®). Subsequently, the films were dried in an oven (Ecoshel 9023A, United States) at 40 °C for 48 hours; then, gently removed and stored in polyethylene bags at room temperature. A control film without bagasse was used for comparison (Table 1).

Table 1. Biocomposite films from native starch of the MMEXV5, MMEXV40, and MMEXCH23 cassava clones, reinforced with three bagasse concentrations.

Biocomposite films	Starch (g)	Bagasse (g)
MMEXV5–B0	5	0
MMEXV5–B1	5	0.05
MMEXV5–B5	5	0.25
MMEXV5–B15	5	0.75
MMEXV40–B0	5	0
MMEXV40–B1	5	0.05
MMEXV40–B5	5	0.25
MMEXV40–B15	5	0.75
MMEXCH23–B0	5	0
MMEXCH23–B1	5	0.05
MMEXCH23–B5	5	0.25
MMEXCH23–B15	5	0.75

Cassava bagasse concentration with particle size between 250–75 μm : B0 = 0% (control), B1 = 1%, B5 = 5% and B15 = 15% by weight of dry starch.

The films were conditioned in a desiccator at 25 °C with 55% relative humidity under a saturated solution of magnesium nitrate hexahydrate ((Mg (NO₃)₂·6H₂O)) for 96 h before each test, following the ASTM E 104, 2002 standard. The films were then removed and placed in dry plastic bags and sealed for 24 hours.

2.3.2. Properties

2.3.2.1. Mechanical analysis

The mechanical analysis was determined following the ASTM D882-02 norm. A universal testing machine, equipped with a 10 N load cell, was used at a 2 mm min⁻¹ speed (Shimadzu AGS-X, Japan). The tensile strength values (σ), elastic modulus (E), and elongation percentage at break (e) of five specimens were recorded. Additionally, the film thickness was recorded on ten measurements of different segments of each sample with a digital micrometer (Mitutoyo, Model 500-196-30, Japan).

2.3.2.2. Thermal analysis

The thermal degradation and stability of the biocomposites were assessed by thermogravimetric analysis (Perkin Elmer TGA7, United States). For this, ~10 mg film samples were heated from 50 to 600 °C at a heating rate of 10 °C min⁻¹ in a nitrogen atmosphere with a 20 mL min⁻¹ flow rate. Weight loss as a function of temperature was plotted as a thermogravimetric analysis curve.

2.4. Statistical analysis

The traits of the native starch (diameter of the granule) and bagasse (distribution of the bagasse particles) were analyzed by descriptive statistics. The biocomposite films' properties were evaluated with an ANOVA and Tukey ($P < 0.05$) means comparison test for a completely randomized design with a factorial arrangement. However, the research's traits were verified for statistical assumptions, analyzing their normal distribution of errors using the Shapiro–Wilk test and homogeneity of the variance using Leven test using the SPSS statistical software [52].

3. Results and discussion

3.1. Starch characterization

Regard the native starch properties, the granules of the MMEXV40 and MMEXCH23 clones were larger, 14.26 ± 2.16 and 14.15 ± 2.01 μm respectively, than those of the MMEXV5 clone, with 12.80 ± 1.92 μm . The diameters were like those reported by Oyeyinka et al. [53]. However, these are considered small [54,55] due to the wide variation of starch in the species (4–40 μm) [56–58].

In Figure 1, the typical Raman spectra of starch are shown, which are associated with the amylose and amylopectin bands, although there is a similarity in the bands' position between the starches of the three cassava clones, they differ in their intensity. It has been mentioned that, in the spectra of the two α -D-glucose polymers, band changes occur, such as the vibrating band of the pyranose ring skeleton around 475–485 cm⁻¹, attributed to the C–O–C ring mode and C–C–O,

likewise, the bands around 320, 410, 769 and 1382 cm^{-1} [59,60]. For amylose, a band has been assigned at 480 cm^{-1} [61], while for amylopectin, there are additional bands at 614, 844, 865, 910, and 1396 cm^{-1} [59,62]. Thus, although amylose and amylopectin almost completely share their spectral characteristics, their different molecular assemblages, and the intensity of their peak are useful for their identification [61–63]. Based on the aforementioned, in the 473 cm^{-1} signals, a greater intensity (height) of the peak was observed for MMEXV40 and MMEXCH23 samples, which shows that these clones have a similarly amylose–amylopectin ratio than the starch of the MMEXV5 clone, an aspect that can cause differences in their size, shape, and granules properties, both, between species and within them [64], and consequently, affect the bioplastics' final properties.

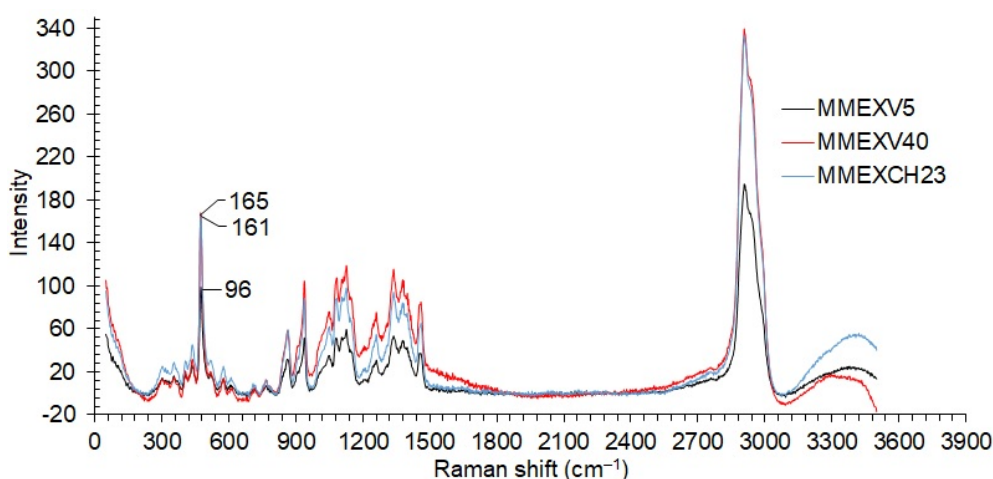


Figure 1. Raman spectra of starch from MMEXV5, MMEXV40, and MMEXCH23 cassava clones.

3.2. Bagasse characterization

3.2.1. Particle size distribution and microstructure

Based on the size of the bagasse particle distribution (Figure 2), a greater quantity of $>250\ \mu\text{m}$ particles (89.9% and 91.79%) was reported. This differs from that stated by Versino and García [34], who reported 56% of $<53\ \mu\text{m}$ particles, probably due to these authors subjecting the fibrous residue to mechanical treatment, a procedure that could cause the fibers to break and thus affect their size and concentration. A similar proportion of particles sizes between each clone's bagasse suggests that the byproduct separation was efficient. Likewise, the results show that, like cassava flour, bagasse is the product of a mixture of various particle sizes [65]. Also, carbohydrates are the largest component in bagasse, mainly starch granules [66], as evidenced in the SEM micrographs (Figure 3), where native starch have very varied forms, including spherical, oval, and truncated, which are characteristic shapes of the species [57,67,68].

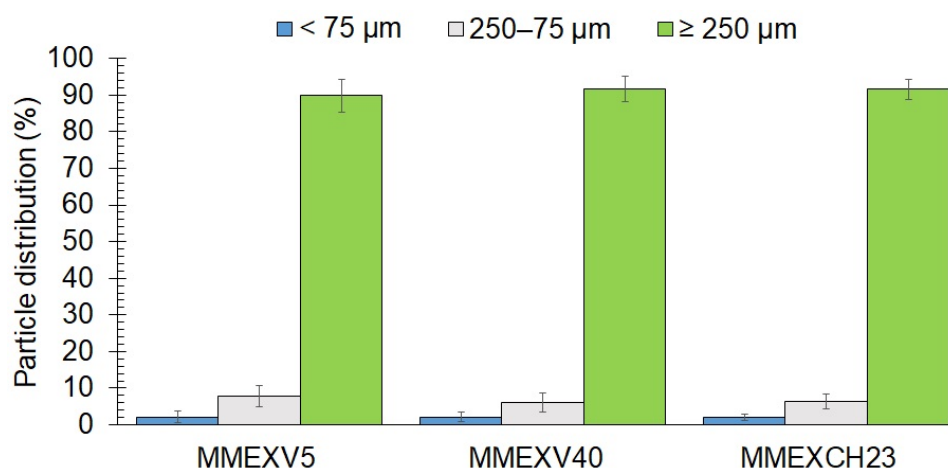


Figure 2. Size of the particle distribution in bagasse of three cassava clones.

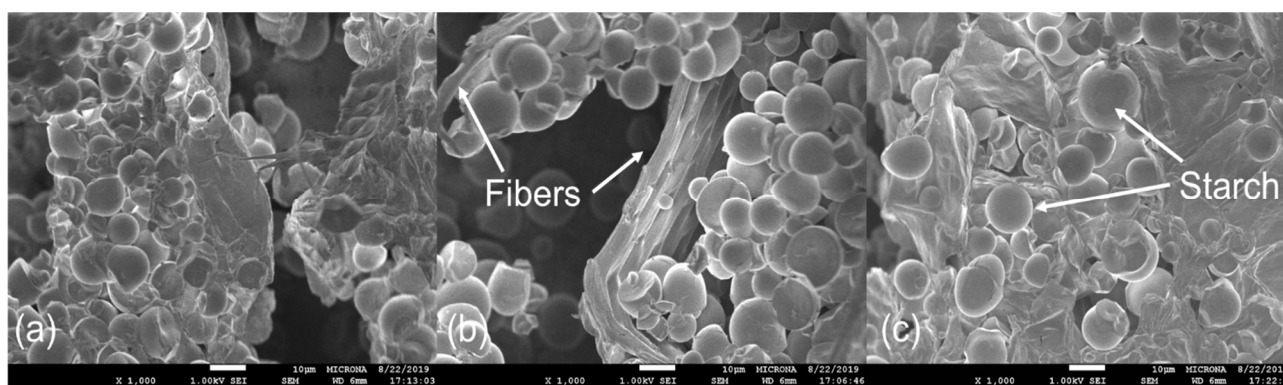


Figure 3. SEM micrographs of the cassava clones bagasse: (a) MMEXV5, (b) MMEXV40, and (c) MMEXCH23 ($\times 1000$).

3.3. Biocomposite film properties

3.3.1. Thickness

Significant differences were found for film thickness in the function of the bagasse concentration ($P < 0.01$) and Clone* Bagasse interaction ($P < 0.05$). In this regard, the thickness of the film increased up to 20.51% in the 15% reinforced films compared to the control (Figure 4b,c). The found 0.29, and 0.40 thicknesses are lower than those obtained by de Azêvedo et al. [69], who reported thicknesses of 0.55 to 0.61 mm and 0.44 to 0.76 mm when using twice corn and potato starch ratios as matrixes respectively, plasticized with 5% glycerol and reinforced with 0.5% to 1.5% silicon particles, while by increasing the amount of plasticizer (7.5%), the thickness increased from 0.71 to 0.77 mm and from 0.54 to 0.75 mm. Therefore, the quantity of the solids presents in the bioplastic films, their botanical origin, and the addition of plasticizers and particles as reinforcement affect their thickness. Also, although no differences were shown for this variable in function of the starch, it was observed that the biocomposites from MMEXV40 and MMEXCH23 were less thick,

compared to those from MMEXV5 (Figure 4a), a clone with lower amylose content (Figure 2). This result concurs with those by Ploypetchara and Gohtani [70], who report greater thickness when using waxy starch from corn and rice, compared to films formulated with normal starch from both species.

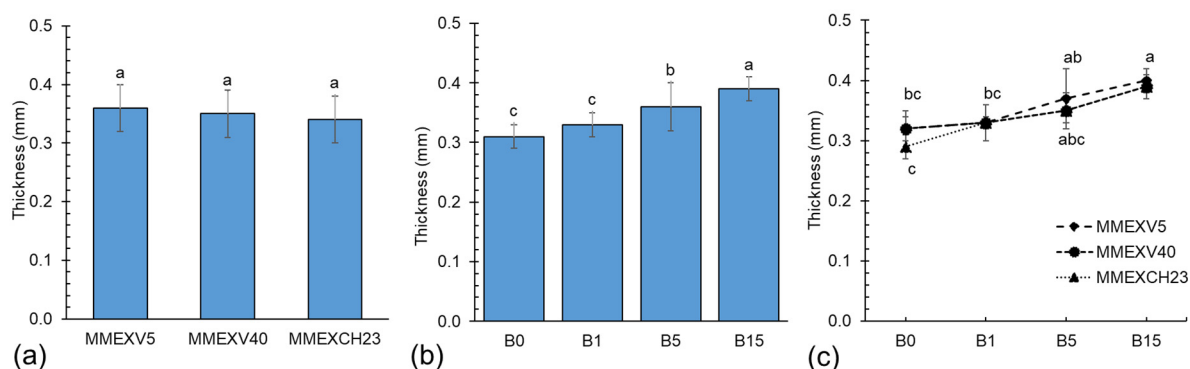


Figure 4. Thickness performance in biocomposite films as a function of: (a) starch source; (b) concentrations of cassava bagasse as reinforcement at B0 = 0%, B1 = 1%, B5 = 5%, B15 = 15%; and (c) Clone*Bagasse interaction. Means with the same letter are not significantly different ($P > 0.05$).

3.3.2. Microstructure

The bagasse presence increased both, the superficial and transverse roughness of the biocomposites, which resulted in heterogeneous surfaces [71], especially at the 15% concentration, compared to lower concentrations and the control. The finding agrees with those by Edhirej et al. [49], who report smooth and homogeneous textures in films with 3% and 6% cassava bagasse with particle sizes of <300 and $300\text{--}600\text{ }\mu\text{m}$. When a high reinforcement proportion is used, the particles density increases, which directly affects the structural integrity of the biocomposites as well as the chemical composition of the lignocellulosic residues. The presence of greater quantity fibers, even at low concentrations, can significantly affect the surface of biocomposites [72]. Likewise, it concurs with the low reinforcement concentrations found by Fazeli et al. [73], who reported smooth films when adding 1% of henequen cellulose nanofibers from *Agave fourcroydes*, to starch polymeric matrixes. On other hand, visually, there were no cracks, pores, or agglomerations (Figure 5). This indicates a good particle dispersion in the polymeric matrix [74] and their structural incorporation due to their biocompatibility [71,75]. This is important, given that dispersion effects matrix properties [2,76]. In this regard, good load incorporation promotes strong interactions between the reinforcement and the matrix, which results in better ductility, resistance, optical properties, among others [51]. However, there are some overtones of unbroken granules; an aspect indicating the possibility of improving the processing conditions.

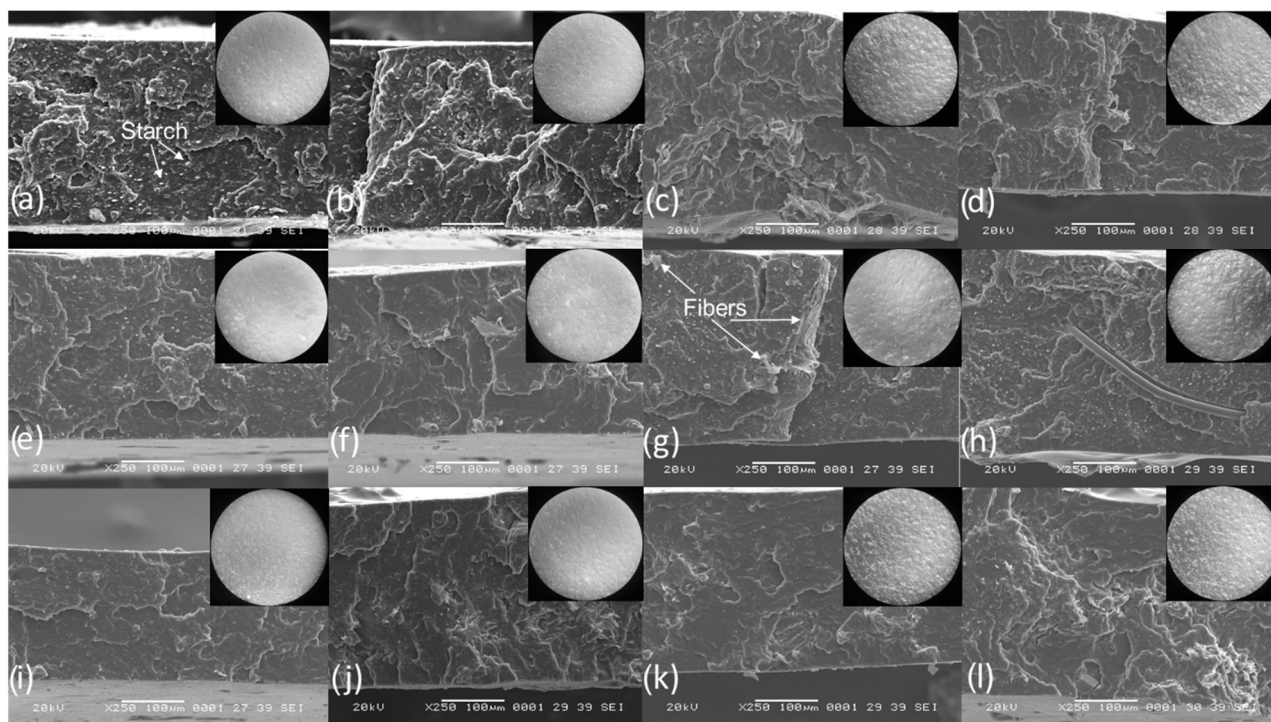


Figure 5. SEM micrographs and stereoscopic images of biocomposites based on thermoplastic starch reinforced with cassava bagasse. MMEXV5, (a) 0%, (b) 1%, (c) 5%, (d) 15%; MMEXV40, (e) 0%, (f) 1%, (g) 5%, (h) 15% and MMEXCH23, (i) 0%, (j) 1%, (k) 5%, (l) 15%.

3.3.3. Mechanical performance

The statistical analysis showed significant differences ($P < 0.05$) in the mechanical properties: σ , E , and e for the different assessed biocomposites due the source of the starch of their polymeric matrix (Figure 6) and the Clone*Bagasse interaction (Figure 7). Still, the bagasse reinforcement only affected E ($P < 0.05$) (Figure 6).

From the abovementioned, the starch of the MMEXV40 clone had better tensile stress and elastic modulus performance, of 2.53 and 60.49 MPa respectively (Figure 6a,b). This performance is probably due to a high content of amylose in the starch granules since the linear chains of this molecule have a high tendency to hydrogen bonds interaction, which forms a rigid rod-shaped filaments network (10 to 30 nm in diameter). Consequently, this provides more rigid, strong, cohesive, and dense films and solutions [77–79]. In contrast, biocomposites made with MMEXV5, which, as expected, achieved a higher elongation at break percentage (37.72%) due to a high amylopectin content (Figure 6c). Lourdin et al. [80], detected a more than 80% increase in tensile strength in bioplastic films made with a high amylose content; compared with greater flexibility in those made from starch with a high amylopectin content [80,81].

At the same time, in the biocomposites with 15% bagasse, a modulus increase of up to 68.90% was recorded, compared to films without reinforcement (Figure 6e). This is because of the rigid nature of the components of the fibrous material [82], such as hemicellulose, cellulose, and lignin, which after starch, are found in greater quantities [83,84]. Furthermore, increasing the concentration

of the reinforcing agent reduces the free volume of the polymeric matrix chains and increases the intermolecular forces. However, mobility is reduced [34,85], which in turn reduces the elongation percentage. Also, due to the fiber's hydrophilic nature, good compatibility between the reinforcement and the matrix is obtained [27]. Overall, the biocomposites reached mechanical values already reported in the literature [71,86]; however, these are considered as low, if they are contrasted with commercial PVC and LDPE films [26].

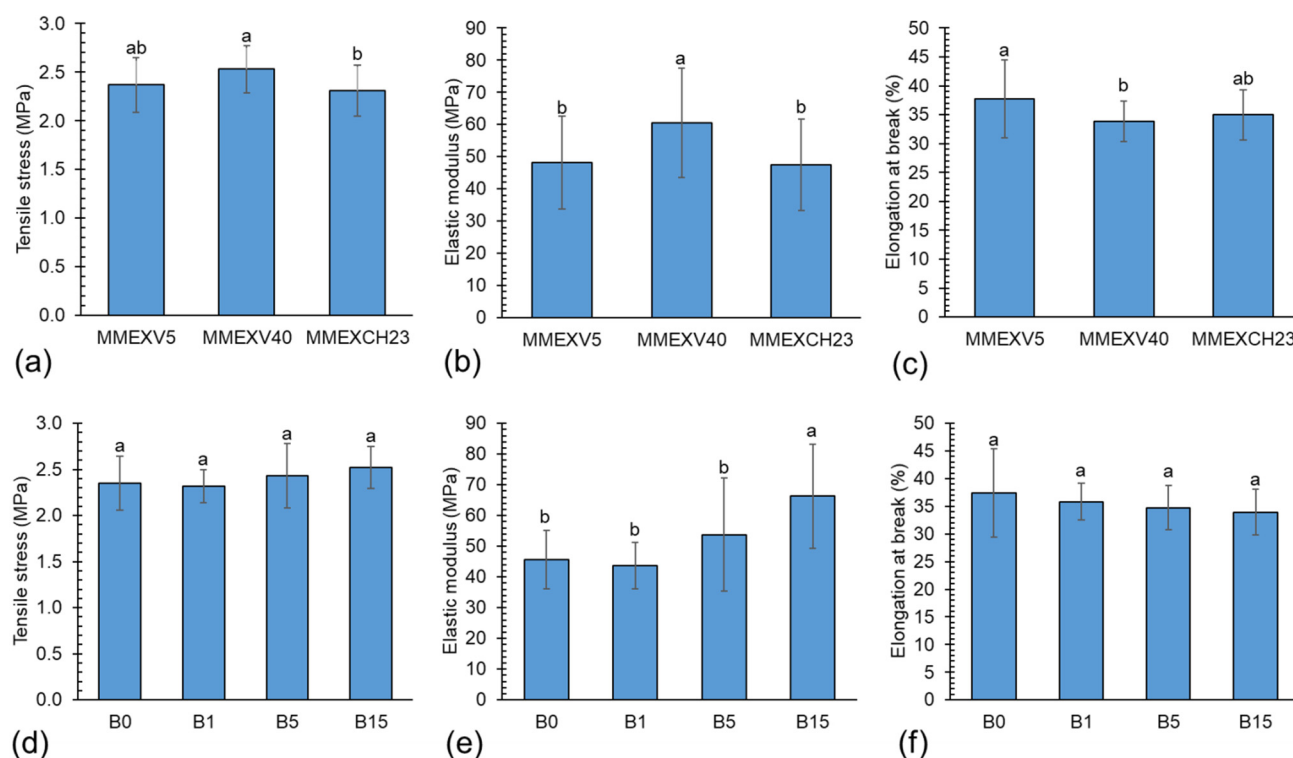


Figure 6. Main effects on the mechanical properties of biocomposite films: (a–c) starch source (MMEXV5, MMEXV40, and MMEXCH23); and (d–f), bagasse concentration as a reinforcing agent of the polymeric matrix. Means with the same letter are not significantly different ($P > 0.05$). Bagasse concentration at B0 = 0%, B1 = 1%, B5 = 5%, and B15 = 15%.

Furthermore, the best combined mechanical performance for tensile stress (Figure 7a) and elastic modulus (Figure 7b) resulted from MMEXV40 and MMEXCH23, mechanical properties that showed a correlation coefficient of 0.83 ($P < 0.01$); however, at a higher concentration (15%), the MMEXV40 biocomposite decreased these properties, contrary to MMEXV5 and MMEXCH23. In this regard, has been reported that the best compatibility between the matrix and the reinforcement (starch, cassava bagasse) was reached at a 6% concentration, independently of the fiber's size, while above this proportion, the films tend to be brittle, with a rough surface with pores and cracks appearance, which are factors that lead to mechanical failures. Nevertheless, given the obtained results, biocomposites could withstand reinforcements of up to 15%, depending on the starch source. Finally, the elongation at break (Figure 7c) performed significantly inverse to tensile stress, by showing a correlation coefficient of -0.48 ($P < 0.01$).

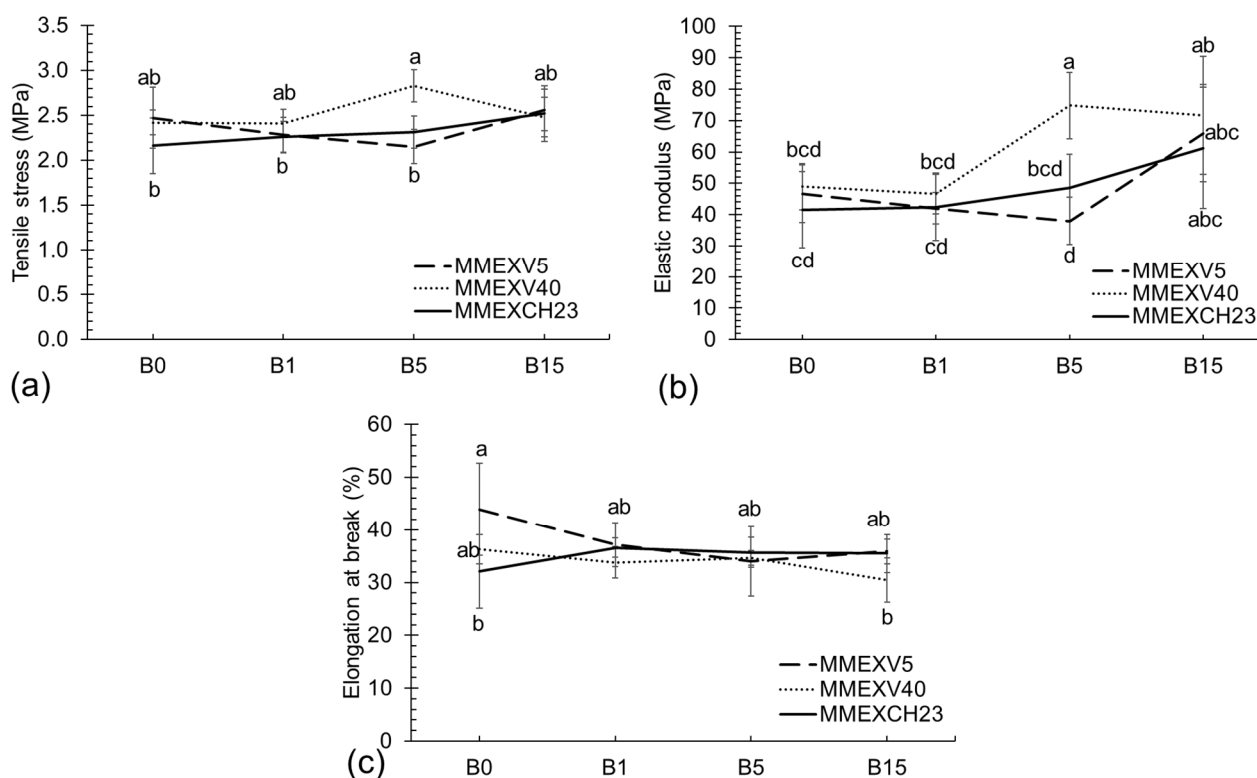


Figure 7. Effect of the Clone*Bagasse interaction on the mechanical properties: (a) stress at break, (b) Young's modulus, and (c) percent elongation at break. Means in the column with the same letter are not significantly different ($P > 0.05$). Bagasse concentration at B0 = 0%, B1 = 1%, B5 = 5%, and B15 = 15%.

From the above, although a low mechanical performance of the biocomposites was achieved, based on tensile strength and elastic modulus, compared with other materials reinforced with cellulose nanofibers [87], and nanocellulose [88–91], and even with other biodegradable polymers, such as polylactic acid [92], the materials obtained can withstand large loads of cassava bagasse as a reinforcing agent (5% to 15%), which leads to savings in the raw material used as matrix, and with it, the possibility of offering added value to the co-product. On the other hand, the biocomposites produced lead to green chemistry [93], by not using chemical agents for the pre-treatment or treatment of bagasse. Meanwhile, elongation at break $>300\%$ higher than that reported by Edhirej et al. [49], suggests that biocomposites can be used for the production of single-use plastics, where high percentages of elongation are required, such as packaging for the food industry.

3.3.4. Thermal analysis

The mass loss curve as a function of TGA temperature shows the thermal degradation of the components of the biocomposite film. The TGA curves revealed a similar performance independently of the starch source (Figure 8). A first mass loss was detected from 50 °C to approximately 145 °C, which relates to the dehydration of the materials [85]. The greatest mass loss occurred between 200–350 °C [76,94], an event that relates to the component's starch (amylose, amylopectin) and glycerol decomposition, from hydrogen groups loss, decomposition and

depolymerization of carbon chains, as well as structural carbohydrates of the bagasse, such as cellulose and hemicellulose [95–97]. The maximum decomposition temperature occurred at $\sim 306^\circ\text{C}$ for all the biocomposites; these results are similar to those by Edhirej et al. [49], even for unreinforced films [86]. In general, the presence of a high proportion of cassava bagasse (5% and 15%) led to a lower mass loss (Figure 8), thus improving the thermal stability of the biocomposites.

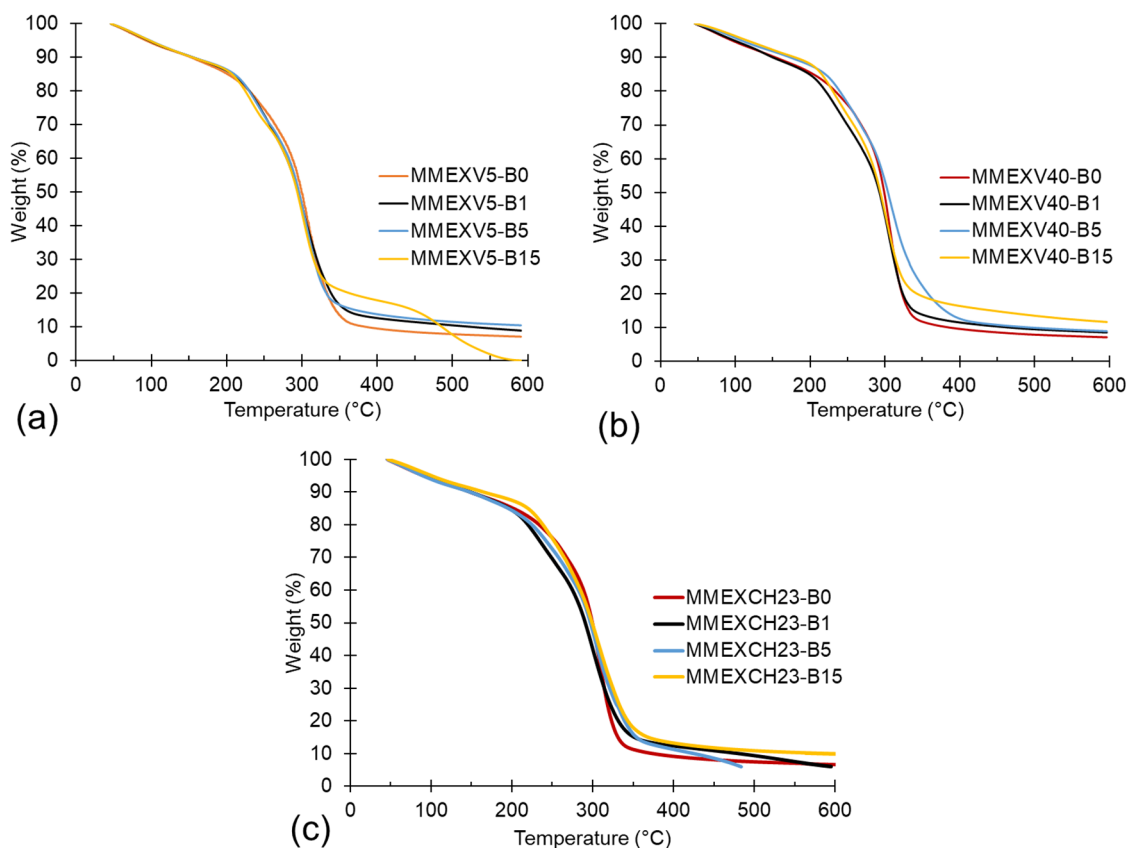


Figure 8. Thermal performance of biocomposite films from: (a) MMEXV5, (b) MMEXV40, and (c) MMEXCH23, reinforced with cassava bagasse at B0 = 0%, B1 = 1%, B5 = 5%, and B15 = 15%.

Based on the obtained results and considering the agro-industrial behavior of the cassava clones in previous research [45], where the MMEXV5 and MMEXV40 clones reached high tuberous roots yield, pulp, peel, fresh and dry bagasse, and starch yield; It is concluded that good agro-industrial performance increases the productive potential of cassava clones to produce bioplastic films, which, together with cassava bagasse as a reinforcing agent of the polymeric matrix, are a sustainable alternative to boost the biorefinery in cassava cultivation [98].

4. Conclusions

In native starch, there are variations in the amylose–amylopectin ratio at the intravarietal level, which contributes to differences in the mechanical properties of biodegradable films. On other hand, cassava bagasse, an underutilized byproduct, can be used as a reinforcing agent for the polymeric

matrixes, thereby helping to decrease industrial waste, offering an added value. From the aforementioned, a 5% bagasse concentration in biocomposites manufacture reported better mechanical performance and thermal stability, which is related to the surface homogeneity of the films. Also, the MMEXV40 cassava clone has the potential to be distributed among farmers due to its high agricultural productivity, which together with its high starch extraction yield and outstanding properties for biocomposite films, make it a promising material for bioplastics production. Finally, it is important to conserve and value the native plant species' germplasm, since they have the potential for unknown uses.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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