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Characterization of cassava starch films plasticized with glycerol and strengthened with nanocellulose from green coconut fibers

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The aim of this study was to obtain and characterize biodegradable films of cassava starch plasticized with glycerol and reinforced with nanocellulose from coconut fibers. The mechanical and physical-chemical properties of the nano-biocomposites films obtained were evaluated. The method used to investigate the viability of incorporating coconut nanocellulose in films was applied through a statistical design of the response surface of 17 formulations containing three independent variables (starch, glycerol and nanocellulose). The films were prepared through casting technique, and the effect of different concentrations of the ingredients in each formulation was investigated by monitoring the dependent variables. The green coconut fiber was composed of 32% cellulose, 38% lignin and 0.25% hemicellulose resulted in nanocellulose with a length (L)/diameter(D) value of 38.9 ± 4.7 after the acid hydrolysis process (64% H_2SO_4 ; 50°C; 10-15 min). The incorporation of nanocellulose resulted in significant changes ($p < 0.05$) in the barrier and mechanical properties. Additionally, there was a significant increase in the Young's modulus and in the tensile of the nano-biocomposites. Consequently, there was a decrease in the percentage of elongation. Thus, films formulated from cassava starch plasticized with glycerol could have significantly altered mechanical, technical and barrier properties due to the incorporation of coconut nanocellulose.

Key words: Biodegradable packaging, cellulose nanocrystals, nano-biocomposites, coconut fibers.

INTRODUCTION

In the last few years there has been a great interest in the development of green technologies around the world for products that have lower environmental impact. Green chemistry, as a whole, involves the development of

chemical processes and products that generate a cleaner, healthier and sustainable environment (Silva et al., 2009). Thus, synthetic plastic materials have received much attention because of their non-biodegradability and

non-renewable sources (Zhong et al., 2012; Meneguín et al., 2017; Chaichi et al., 2017).

One solution found to improve the environmental impact of synthetic plastics was the development of biomaterials from renewable polymers that can substitute synthetic materials. Starches are polymers with a high potential to produce flexible films and are inexpensive, biodegradable and highly available from renewable sources. However, the primary challenge is to substitute conventional packages while maintaining the same efficacy, quality and shelf-life. These results can be obtained through the control of mechanical properties and permeability (Seligra et al., 2016; Henrique et al., 2007; Qazanfarzadeh and Kadivar, 2016; Montero et al., 2017).

Ligno-cellulosic fibers are excellent raw materials for polymer and composites chemistry, which can be verified by the high number of patents and products already being commercialized with these fibers (Kermit, 2010; Ortega and Baillie, 2011; Sohei et al., 2011; Kun, 2011). Cellulose present in ligno-cellulosic fibers is formed by amorphous regions appearing as imperfections on microfibrils and by crystalline regions. The cellulose fibrils can be cleaved transversally when hydrolyzed in an acid medium, resulting in small crystals. These highly crystalline particles are referred to as nanocellulose, micro-crystalline cellulose, cellulose cristalito, nanocrystals of cellulose, whiskers or cellulose nanowhiskers when the L/D ratio is lower than 200 (Costa et al., 2016; Niu et al., 2017; Sun et al., 2017).

In recent years, various research groups have reported new techniques for the formation of nanocellulose from different natural sources (Myllytie et al., 2010; Rosa et al., 2010; Silva et al., 2012; Costa et al., 2014; Machado et al., 2014; Rusmirović et al., 2017; Wang et al., 2017), and the incorporation of nanocellulose into polymeric materials. Nanocellulose crystals have numerous advantages compared with other nano-structured materials, such as ease of formation, low cost of raw materials, diverse characteristics, which are dependent on the source of the natural substrate, and more beneficial mechanical properties compared with carbon nano-tubes and inorganic nano-fibers (Strucova et al., 2006; Panaitescu et al., 2015; Qin et al., 2016; Li et al., 2016; Liu et al., 2017).

This work evaluates the incorporation of the nanocrystals nanocellulose from green coconut fibers in polymeric films produced with cassava starch and plasticized with glycerol.

MATERIALS AND METHODS

The following materials were used for this study: cassava starch

(donated by Cargill Agricola SA), glycerol (Synth), coconut fiber (composed of 38% lignin and 32% cellulose) provided by Embrapa Agroindustria Tropical (Fortaleza, CE, Brazil). In addition, reagents such as sodium hydroxide, sodium hypochlorite, sulfuric acid (98.08%), acetic acid (Vetec) and cellulose membrane (D9777 – 100 FTO) for dialysis were obtained from Sigma-Aldrich.

Extraction of cellulose from coconut fiber

The method used to extract cellulose was adapted from previously published method by Rosa et al. (2010), Samir et al. (2005) and Machado et al. (2014). The coconut fibers were oven-dried in circulation air at 35°C for 5 h, crushed to obtain a fine particulate and sifted using a 40-mesh sieve. The samples (30 g) were then washed with a solution of NaOH 2% (1.200 mL) for 4 h under constant agitation at 80°C. The resulting solution was filtered and washed in water to obtain the pulp. The washing process was repeated four times to completely remove the water-soluble materials. After washing the fibers, the pulp delignification process was performed by bleaching, using a mixture of 1.7% sodium hypochlorite (300 mL) and a buffer solution (300 mL). The resulting solution was constantly agitated at 80°C for 6 h. Then, the solution was filtered and oven-dried in circulation air at 25°C for 10 h to obtain the cellulose. Finally, the cellulose was pulverized in a mill (Cadense Ltda - Brazil).

Preparation of films strengthened with green coconut cellulose

The films were processed using a casting technique, which consisted of the preparation of a filmogenic solution by dissolving the cassava starch in distilled water (3 to 6 g/100 g) and using glycerol as a plasticizer agent (0.5 to 2.5 g/100 g). Thereafter, a dispersion of nanocellulose (0.1 to 0.5 g/100 g) was added, which was previously heated to the starch's gelatinization temperature (70°C) under constant manual agitation. To prepare the filmogenic solution with approximately (40 g in mass), polystyrene Petri dishes were used. They were then dehydrated in a kiln with air circulation (35±2°C - Temperature range was used to avoid melting the petri dish and burning the film-forming solution) for 18 to 20 h (time required for complete drying). The obtained films were stored (60% relative humidity, 25°C) in a desiccator with a saturated sodium chloride solution for 10 days before being characterized (Veiga-Santos and Scamparini 2004). Seventeen formulations were prepared according to a Central Composite Rotational Design (DCCR 2³) (Table 1).

Characterization of nanocellulose and nano-biocomposites

Transmission Electronic Microscopy (TEM)

The coconut fiber nanocellulose solution was analyzed by transmission electron microscopy (TEM) to determine the length (L) and diameter (D) of the fibers and to indicate the state of crystal aggregation. The nanocellulose solution was mixed in equal volumes with 2% aqueous uranyl acetate (UA). In total, 10 ml of this mixture was poured in a 400 copper mesh and left to stand for 30-60 s. The mesh was dried and examined on a scanning electron microscope CM12-transmission (STEM) operating in a bright field mode at 80 kV. The lengths and diameters of the crystals were measured directly from the transmission electronic micrographs

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Table 1. Real and codified values from the Central Composite Rotational Design (DCCR) of the independent variables: cassava starch (g/100 g; X_1), glycerol (g/100 g; X_2) and coconut cellulose (g/100 g; X_3).

Formulations	Codified Values			Real values (g/100 g)		
	Cassava starch (X_1)	Glycerol (X_2)	Nanocellulose (X_3)	Cassava starch	Glycerol	Nanocellulose
F1	-1.00	-1.00	-1.00	3.60	0.90	0.18
F2	-1.00	-1.00	1.00	3.60	0.90	0.42
F3	-1.00	1.00	-1.00	3.60	2.10	0.18
F4	-1.00	1.00	1.00	3.60	2.10	0.42
F5	1.00	-1.00	-1.00	5.40	0.90	0.18
F6	1.00	-1.00	1.00	5.40	0.90	0.42
F7	1.00	1.00	-1.00	5.40	2.10	0.18
F8	1.00	1.00	1.00	5.40	2.10	0.42
F9	-1.68	0.00	0.00	3.00	1.50	0.30
F10	1.68	0.00	0.00	6.00	1.50	0.30
F11	0.00	-1.68	0.00	4.50	0.50	0.30
F12	0.00	1.68	0.00	4.50	2.50	0.30
F13	0.00	0.00	-1.68	4.50	1.50	0.10
F14	0.00	0.00	1.68	4.50	1.50	0.50
F15*	0.00	0.00	0.00	4.50	1.50	0.30
F16*	0.00	0.00	0.00	4.50	1.50	0.30
F17*	0.00	0.00	0.00	4.50	1.50	0.30

*Central points.

using Image Tool 6.3 (Media Cybernetics, Inc., Bethesda, MD) with 30 measurements to determine the average values and standard deviation (Silva et al., 2012).

Mechanical properties

The tensile test of the nano-biocomposites were conducted using an eletromechanical universal testing machine by EMIC (model DL2000/700) with maximum load of 20 KN following ASTM D-882 (2001) with velocity of 12.5 mm min^{-1} at 25°C . Eight proof bodies were used for each sample to get average value. The proof bodies were of length 80 mm and width 25 mm, and their strength was calculated by dividing the maximum applied force by the area of the film (width \times thickness). The strain at breakage was calculated by dividing the final length by the projection of the probe tip (50 mm) and multiplying by 100 (Fakhouri et al., 2013).

Thickness (T)

The film thickness was determined by an average thickness from 6 measurements in random positions using a digital flat-headed micrometer (Mitutoyo; resolution $1 \mu\text{m}$) in triplicate.

Water activity (aw) and humidity

The water activity (a_w) of the films was measured with a decagon (AQUALAB LITE). The film's humidity was determined by drying using an infrared (Mettler) drying unit (LTJ) by adjusting the radiation intensity emitted until the sample reached 110°C (Veiga-Santos et al., 2005). The analyses were performed in triplicate.

Permeability to water vapor (PWV)

The water vapor permeability of the films was performed through a gravimetric method, recommended by ASTM E96-00 modified (2000). The standard method consisted of successively weighing a capsule with a hermetically sealed surface using the film. The samples were stored with a desiccator substance (silica gel) in its interior and placed in a humidity-controlled environment (desiccator at room temperature $\pm 23^\circ\text{C}$ and 70% relative humidity, using sodium chloride). Water vapor transport (WVT) was determined from the weight gain of the permeation, measuring over 24 h for 10 days. The permeability to water vapor was calculated according to Equation 1 (Famá et al., 2012):

$$\text{Permeability to water vapor} = \text{WVT} \cdot e / \text{Po} \cdot \text{RH} \quad (1)$$

Where, e is the film thickness and Po is the saturation vapor pressure of water at room temperature (Gennadios et al., 1994).

Central composite rotational design (DCCR) and statistical analysis

The films were developed using a central composite rotational statistical design with a 2^3 model containing 4 axial points, 10 orthogonal points and 3 central points, which totaled 17 formulations. The real and the codified values of the independent variables, cassava starch (% m/m; X_1), glycerol (% m/m; X_2) and coconut nanocellulose (% m/m; X_3), which were defined with a process restriction, can be found in Table 1. To evaluate the influence of nanocellulose on the properties of nano-biocomposites, a film of cassava starch without the nanocellulose was used as control and was composed of 4.5% cassava starch and 1.5% glycerol.

The data were treated using a Tukey Test and ANOVA to identify whether the alterations in the evaluated parameters were significant at a 95% significance level. To evaluate the influence of the independent variables, the response surface methodology was used. The second degree polynomial was calculated using the program Statistic 7.0 (Stat Inc, Minneapolis, MN, USA) to evaluate the influence of the independent variables (X_1 , X_2 and X_3) on each dependent variable (Y) according to the model generated below (Equation 2):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

Where, Y = dependent variable; X_1 , X_2 and X_3 = independent variables; b_0 = compensation term; b_1 , b_2 and b_3 = linear terms; b_{11} , b_{22} and b_{33} = quadratic terms; and b_{12} , b_{13} and b_{23} = interaction terms among the independent variables.

RESULTS AND DISCUSSION

Characterization of nanocellulose from green coconut fibers

Chemical processes are the primary methods for cellulose nanocrystals isolation from ligno-cellulosic fibers. These methods generally involve washing the fibers with alkaline solutions and bleaching to obtain cellulose, followed by acid hydrolysis using strong acids.

These hydrolysis processes rely on the crystalline regions being insoluble in acids in certain conditions, which is due to the inaccessibility presented by the high organization of the cellulose molecules in their nanostructure. Conversely, the natural disorganization of the cellulose molecules in the amorphous regions favors the accessibility of the acids and consequently the hydrolysis of the cellulose chains present in these regions. Therefore, the isolation of nanocellulose is facilitated by the faster hydrolysis kinetics shown by amorphous regions compared with crystalline regions (Samir et al., 2005).

After four successive phases of treatment with an alkaline solution, the fibers were bleached to intensify the effects of the alkaline treatment, to remove the residual lignin and to increase the exposure of cellulose. After bleaching, the fibers changed from brown to white, as observed in the alkaline treatment, indicating that the bleaching had produced the expected results. For each 10 g of fiber that was washed and bleached, approximately 1.25 g of cellulose was obtained with a 12.5% yield for this extraction from this cellulose; the yield obtained for the production of nanocrystals was 66%.

The cellulose nanocrystals were prepared in an aqueous dispersion (0.033 g/10 mL). Various studies have described the use of faster hydrolysis time periods for obtaining nanocellulose solutions, compared with those used in this study. This indicated that the quantity and percentage of acid as well as the temperature and vigorous agitation all significantly contributed to

optimizing the extraction process because the nanocellulose solution was obtained after a short period of hydrolysis.

Rosa et al. (2010) prepared nanocellulose from green coconut fibers (the same matrix used in this study) in faster time frames (120, 150 and 180 min) using the same concentration of H_2SO_4 (64% v/v); however, a lower temperature (45°C) and a smaller proportion of cellulose pulp and acid (10 g/10 ml) was used. Therefore, the method used in the present study (12 ml/g cellulose, 50°C, 10 to 15 min heating) reduced the time for obtaining nanocellulose by a factor of 10 (much shorter time). Silva et al. (2012) also prepared nanocellulose by hydrolysis with H_2SO_4 (64% v/v) from 12 ml/g cellulose pulp from piassaba with constant agitation for 15 to 17 min at 50°C. The acid concentration, temperature and ratio of acid to cellulose pulp used in this study were the same as in the Silva study, which demonstrated that these conditions were favorable for obtaining nanocellulose solutions with a shorter hydrolysis period.

Figure 1 shows the micrographs obtained by the transmission electronic microscopy (TEM) of the nanocrystals from coconut cellulose in an aqueous solution (0.66 g/10 ml). These images provide evidence of the efficiency of the acid hydrolysis treatment to obtain nanocellulose from green coconut fiber. The images also confirm that the aqueous dispersions contain nanocrystals, which primarily consist of individual and some aggregate fibrils. This was consistent with the structural model proposed by Battista and Smith (1962) and with the results obtained by Samir et al. (2005), Rosa et al. (2010), Thomas et al. (2015), Rambabu et al. (2016) and Silva et al. (2012).

The L and D of the cellulose crystals and the relationship of L/D are listed in Table 2. The conditions of hydrolysis used for the preparation of the nanocrystals affected the dimensional properties of these particles (Bondeson et al., 2006). This was because the prolonged acid attack could destroy the amorphous parts of the cellulose and destroy the partially crystalline zones of the fibers, which resulted in a reduction of the nanocrystal length.

The L of the coconut cellulose nanocrystals varied between 98 to 430 nm, and the average D was 6 nm. The average L/D ratio was 38.9 ± 4.7 , which was in a good range for use as a support in nano-biocomposites (Rosa et al., 2010). The coconut cellulose nanocrystals obtained with longer hydrolysis time and a lower temperature by Rosa et al. (2010) had an average L and D of 197 and 5.8 nm, respectively, and a L/D ratio of 39. The results found in this study were also consistent with those found by other authors, who characterized the dimensions of nanocellulose from different ligno-cellulosic sources (Bondeson et al., 2006; Elazzouzi-Hafraoui et al., 2008; Roohani et al., 2008; Silva et al., 2012; Rambabu et al., 2016). Methods used to prepare nanocellulose are inexpensive; in addition, green coconut bark is widely

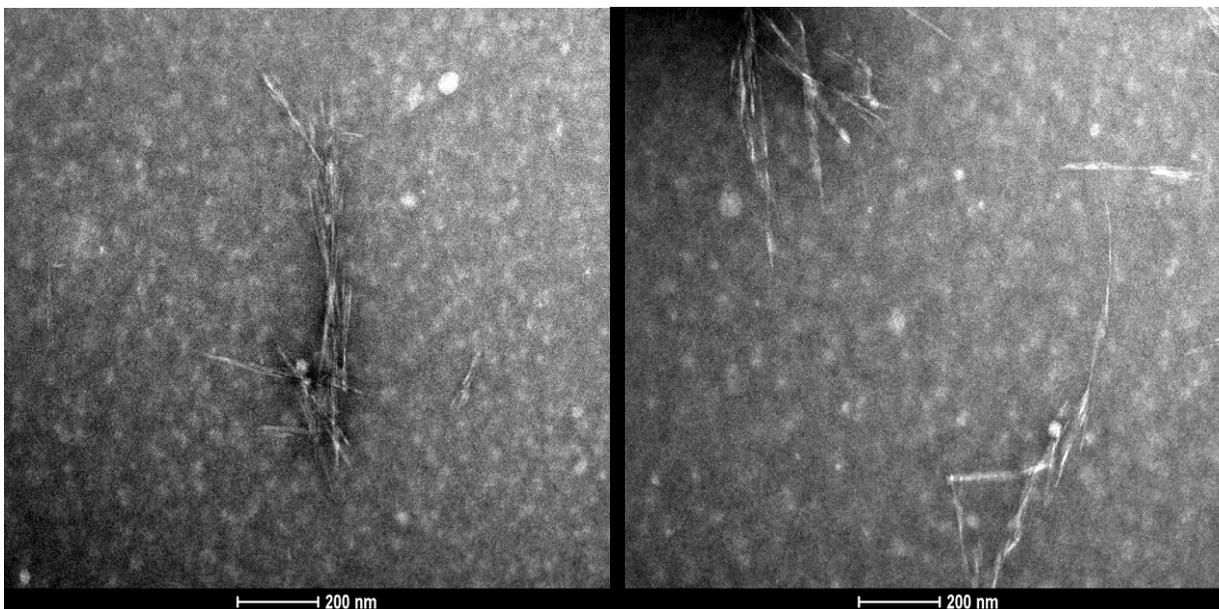


Figure 1. Micrographs obtained (TEM) from the coconut nanocellulose solution, evidencing the presence of nanocrystals (Scales: 200 nm).

Table 2. Dimension of the crystals of nanocellulose from green coconut fibers prepared by acid hydrolysis determined from TEM images.

Length (L –nm)			Thickness (D – nm)	Rate (L/D)	
Minimum	Maximum	Average		Interval	Average
98	430	201±57	5.6±0.98	18.2 – 75.4	38.9±4.7

available, and its disposal is a problem. Thus, green coconut bark can be used as a viable raw material for the preparation of nanocellulose and as a support for nano-biocomposites. Perhaps, the only limitation of the method used is the use of strong acids to obtain the nanocrystals.

Barrier properties of nano-biocomposites

The films were prepared from the values established by the statistical design. Despite drying the 40 g of filmogenic solution in Petri dishes, the resulting thicknesses varied (0.093 mm for formulation F9 to 0.146 mm for formulation F10). Therefore, there were few significant differences between the nano-biocomposite samples ($p < 0.05$) (Table 3). The independent variables (percentage of cassava starch, glycerol and nanocellulose) exerted significant effects ($p < 0.05$) on the thickness (dependent variable) of the films. As expected, the percentage of starch present in the formulation was primarily responsible for the increase in thickness of the nano-biocomposites. Thus, the differences in film thickness are principally due to the higher solids

concentration of the solution.

Water activity (a_w) and water content is critical factor for the stability of food. The values of a_w varied from 0.638 to 0.710 among the 17 formulations considered in this study, which were lower than the control (0.830). Therefore, these formulations were considered products with intermediate humidity (using classification for foods). According to Table 3, all films that contained coconut nanocellulose showed a significant decrease in the humidity and a_w level when compared with the control film. Formulation F11 had the lowest a_w (0.638 ± 0.07) and the lowest humidity percentage (15.50 ± 1.22) values. Silva et al. (2012) formulated and characterized starch films containing nanocellulose from eucalyptus and found a_w values varying from 0.461 to 0.630. They also verified that the formulations containing the higher percentages of nanocellulose had lower a_w values.

The results indicated that the (a_w) of starch films plasticized with glycerol can be controlled and improved through the incorporation of nanocellulose from green coconut fibers obtained by acid hydrolysis. Therefore, the addition of these nanocrystals can considerably increase the shelf life of nano-biocomposites through a reduction

Table 3. Average values (\pm sd – standard deviation) of the dependent variables regarding the barrier properties of the nano-biocomposites and control (C).

F	T \pm sd	aw \pm sd	TS \pm sd	H \pm sd	PWV \pm sd
Control	0.079 \pm 0.03 ^a	0.830 \pm 0.21	57.36 \pm 1.34	42.64 \pm 2.18	9.7 \times 10 ⁻⁸ \pm 0.31
F1	0.095 \pm 0.01 ^a	0.653 \pm 0.18 ^a	84.00 \pm 0.92 ^{a,e,h}	16.00 \pm 1.22	6.35 \times 10 ⁻⁸ \pm 0.67 ^{a,g}
F2	0.099 \pm 0.02 ^{b,e,f}	0.657 \pm 0.18 ^a	82.64 \pm 1.07 ^{a,e,h,i}	17.36 \pm 1.22	5.98 \times 10 ⁻⁸ \pm 0.87 ^{b,c,d,e,f,i}
F3	0.107 \pm 0.02 ^{b,f}	0.710 \pm 0.12	68.99 \pm 1.33	31.01 \pm 2.07	6.33 \times 10 ⁻⁸ \pm 0.89 ^a
F4	0.125 \pm 0.03 ^{c,h}	0.692 \pm 0.03 ^b	73.14 \pm 1.77 ^d	26.86 \pm 1.80 ^a	6.01 \times 10 ⁻⁸ \pm 0.74 ^{b,d,f,i}
F5	0.129 \pm 0.02 ^c	0.643 \pm 0.10 ^c	80.13 \pm 0.89 ^{b,ef,g,h}	19.87 \pm 1.21	6.30 \times 10 ⁻⁸ \pm 0.71 ^a
F6	0.135 \pm 0.01 ^g	0.654 \pm 0.09 ^a	80.69 \pm 1.13 ^{b,ef,g,h,i}	19.31 \pm 1.39	5.99 \times 10 ⁻⁸ \pm 0.75 ^{b,c,d,e,f,i}
F7	0.145 \pm 0.03 ^d	0.691 \pm 0.05 ^b	77.88 \pm 1.09 ^{c,f}	22.12 \pm 2.05	6.32 \times 10 ⁻⁸ \pm 0.60 ^a
F8	0.146 \pm 0.01 ^d	0.674 \pm 0.06 ^e	77.50 \pm 1.34 ^c	22.50 \pm 1.19	6.03 \times 10 ⁻⁸ \pm 0.68 ^{b,f}
F9	0.093 \pm 0.02 ^{e,f}	0.693 \pm 0.11 ^b	73.80 \pm 2.01 ^d	26.20 \pm 2.18	5.95 \times 10 ⁻⁸ \pm 0.76 ^{c,d,e,f,i}
F10	0.146 \pm 0.03 ^d	0.685 \pm 0.10 ^{d,f}	81.22 \pm 1.85 ^{e,g,h,i}	18.78 \pm 1.49 ^b	5.97 \times 10 ⁻⁸ \pm 0.86 ^{d,e,f,i}
F11	0.097 \pm 0.02 ^f	0.638 \pm 0.07 ^c	84.50 \pm 1.75	15.50 \pm 1.22	5.94 \times 10 ⁻⁸ \pm 0.93 ^{e,f,h,i}
F12	0.139 \pm 0.01 ^g	0.693 \pm 0.09 ^b	71.17 \pm 1.62	28.83 \pm 1.09 ^a	5.98 \times 10 ⁻⁸ \pm 0.77 ^{f,i}
F13	0.119 \pm 0.03 ^{h,i,j,l}	0.680 \pm 0.10 ^{d,e,f}	79.42 \pm 1.55 ^{f,g}	20.58 \pm 1.12	6.40 \times 10 ⁻⁸ \pm 0.66 ^g
F14	0.122 \pm 0.02 ^{h,i,l}	0.676 \pm 0.12 ^{e,f}	80.39 \pm 1.19 ^{g,h,i}	19.61 \pm 0.18	5.89 \times 10 ⁻⁸ \pm 0.91 ^h
F15*	0.117 \pm 0.02 ^{i,j,l}	0.683 \pm 0.15 ^{d,f}	81.21 \pm 1.33 ^{h,i}	18.79 \pm 1.45 ^b	5.97 \times 10 ⁻⁸ \pm 0.81 ⁱ
F16*	0.116 \pm 0.01 ^{j,l}	0.680 \pm 0.05 ^f	81.92 \pm 1.32 ^j	18.08 \pm 1.60	5.94 \times 10 ⁻⁸ \pm 0.86 ⁱ
F17*	0.119 \pm 0.03 ^l	0.682 \pm 0.08 ^f	81.72 \pm 1.24 ^{h,i}	18.28 \pm 1.85	5.93 \times 10 ⁻⁸ \pm 0.81 ⁱ

Formulations (F) of DCCR; *Central Points. Values presenting the same letter, in the same column, do not show significant differences ($p > 0.05$) by the Tukey Test at 95% confidence level. T, thickness - mm; aw, water activity; TS, total solids - %; H, humidity (%); PWV, permeability to water vapour - gH₂O/m.s.Pa.

in water quantity available for microorganism growth and for the occurrence of chemical reactions.

Total solids, humidity and water activity were significantly affected ($p < 0.05$) by three independent variables. The plasticizer glycerol exerted the most significant effect ($p < 0.05$) on three parameters. It had a negative effect on the total solids content and a positive effect on the humidity and aw values. Glycerol is a hydrophilic plasticizer that interacts with water to form hydrogen bonds, thus interfering with values of the aw and humidity of nano-biocomposites.

When glycerol was used under low concentrations, the films showed low values of water activity and humidity. The anti-plasticizing effect has already been reported by other authors when evaluating this plasticizer in different combinations and with other types of starch (Gaudin et al., 2000; Chang et al., 2006; Mali et al., 2008; Liu et al., 2013). Balakrishnan et al. (2017) evaluated potato starch films with pineapple nanocellulose, from the results, it was assumed that the starch glycerol system exhibits a heterogenous nature and cellulose nanofibers tend to move towards glycerol rich starch phase. Barrier properties also improved with the addition of nanocellulose up to 3 wt% but further addition depreciated properties due to possible fiber agglomeration. Formulations F1, F2, F5 and F6 containing 0.9% of the plasticizer, and F11, with 0.5%, showed lower values of humidity and aw when compared with the other

formulations and the control.

The incorporation of nanocellulose in the polymeric matrix of cassava starch with glycerol resulted in a decrease in the values of water vapor permeability, which varied from 5.89 \times 10⁻⁸ to 6.40 \times 10⁻⁸ gH₂O/m.s.Pa. This was much lower than the control (9.7 \times 10⁻⁸ gH₂O/m.s.Pa) (Table 3). Formulation F13, which had a lower percentage of nanocellulose (0.1% m/m), showed the higher permeability among the nano-biocomposites studied (6.40 \times 10⁻⁸ gH₂O/m.s.Pa), whereas F14, which had the highest percentage of nanocellulose (0.5% m/m) showed the lower value (5.89 \times 10⁻⁸ gH₂O/m.s.Pa). Therefore, there was a decrease of 34 and 39.3% in the water vapor permeability for F13 and F14, respectively, compared with the control film. The presence of the cellulose nanocrystals in the matrix in any concentration promoted a reduction in the water vapor permeability, which led to alterations in the nano-biocomposite structures. This reduction in water vapor permeability occurs because the nanocrystals, when associated with glycerol, act as a barrier and decrease the free spaces in the polymeric matrix and impair the passage of vapor (Rosa et al., 2010; de Mesquita et al., 2011; Müller et al., 2008). Besides that, it is supposed that nanocrystals interact through hydrogen bridges with starch chains. Similar results regarding the decrease in water vapor permeability have been determined by Azeredo et al. (2009) when incorporating nanocrystals of commercial cellulose in

mango puree films. Thus, coconut cellulose nanocrystals used as a physical barrier to water permeability can be explained by the high degree of crystallinity of the nanoparticles, which present a crystallinity index of approximately 70% (calculated through the analysis of X-ray diffraction – DRX – data not shown). The crystallinity of the cellulose nanoparticles have also been used to explain the decrease in the water vapor permeability rate and water solubility in PWV membranes (Paralikara et al., 2008), Xylan membranes (Saxena and Ragauskas, 2009) and starch films plasticized with sucrose and inverted sugar (Silva et al., 2012) incorporated with nanocrystals. Water transport in edible films based on hydrophilic materials such as starch, is a complex phenomenon due to the strong interaction of sorbed water molecules with the polymeric structure. Slavutsky and Bertuzzi (2014) identified that the measured film solubility, contact angle, and water sorption isotherm indicated that reinforced starch/nanocellulose films have a lower affinity to water molecules than starch films. The interaction between nanocellulose and starch chain is favoured by the chemical similarities of both molecules.

The statistical study showed that the independent variables exerted a significant effect ($p < 0.05$) based on the concentration of nanocellulose (L and Q), glycerol (Q) and starch (Q) on the PWV (permeability to water vapor) of the nano-biocomposites, which were represented by quadratic and linear functions. The concentration of coconut nanocellulose directly influenced the permeability to water vapor; as the concentration of these nanoparticles increased in nano-biocomposites, the permeability to water vapor decreased ($R^2 = 0.98$) (Figure 2). Similar results to this study were found by Lu et al. (2005), Wang et al. (2006) and Cao et al. (2008) for nano-biocomposites with starch, protein and starch matrixes, respectively, containing nanocellulose from cotton linter (Lu et al., 2006) and from hemp (Cao et al., 2008).

The analysis of the experimental data for the different formulations resulted in polynomial second order equations for each response surface generated: thickness (T), total solids (TS), humidity (H), water activity (a_w) and permeability to water vapor (PWV) (Table 4). Through the use of these equations, it is possible to optimize each independent variable concentration to obtain a better response in relation to the barrier properties, assisting future studies.

Mechanical properties of the films

The incorporation of a coconut nanocellulose solution to biodegradable films of cassava starch plasticized with glycerol in different concentrations resulted in alterations of the mechanical properties of all formulations studied (Table 5). The coconut cellulose nanocrystals, combined with other independent variables were efficient in

increasing the maximum tension. An increase of up to 1.619% (F11) was observed compared with the control (starch film without nanocellulose). This property was significantly altered ($p < 0.05$) with the incorporation of nanocellulose in all concentrations studied (0.1 to 0.5%).

The value of the Young's modulus (elasticity) also increased significantly ($p < 0.05$) with the incorporation of nanocellulose. Formulations F10, F6 and F11 showed an increase of 3.419, 5.525 and 47.090%, respectively, in this parameter compared with the control film. However, there was a decrease of deformation in the rupture (ϵ) of all of the formulations containing coconut nanocellulose, which was expected. This was most likely due to the increase in rigidity of the nano-biocomposites, which showed a decrease in the ductile capacity of the nanomaterial. This behavior is usually expected when a more rigid component – in this case the nanoparticles of cellulose – is added to a more flexible material (the starch).

This effect can be attributed to the phenomenon known as the mechanical percolation of cellulose nanocrystals and the formation of a continuous network of nanocellulose linked by hydrogen interactions. Alternatively, it could have been due to a good dispersion of the nanocrystals in the matrix, which would indicate a good interaction among the components of the film that could be proven with images of nanoparticle distribution inside the film by electron microscopy. Samir et al. (2005) have reported that cellulose nanocrystals are regions that grow under controlled conditions, which allow for the formation of individual crystals of high purity. This highly ordered structure could show high resistance and significant changes in some important properties of the materials, such as electrical, optical, magnetic, ferromagnetic, dielectric and conductivity.

Chaichi et al. (2017) developed edible pectin film reinforced by crystalline nanocellulose and the incorporation of three levels of nanocellulose (2, 5 and 7% w/w) on mechanical and water vapor barrier properties of pectin-based biodegradable film were investigated. The optimum result was obtained through the nanocomposite film with 5% nanocellulose in terms of mechanical and water vapor properties as the tensile strength increased up to 84% and water vapor permeability decreased by 40%. Cao et al. (2008) reported similar results to this study when they formulated and mechanically characterized biodegradable films of thermoplastic starch and nanocellulose from hemp fibers as a support material. The resistance (maximum tension) increased from 3.9 to 111.5 MPa when the content of nanocellulose increased from 0 to 30%. For the same concentrations of nanocellulose, the Young's modulus increased from 31.9 to 823.9 MPa, respectively. Wang et al. (2010) developed starch nanocomposites and polyurethane with varied concentrations of nanocellulose through casting and reported that the incorporation of 1% nanocellulose in the matrix increased

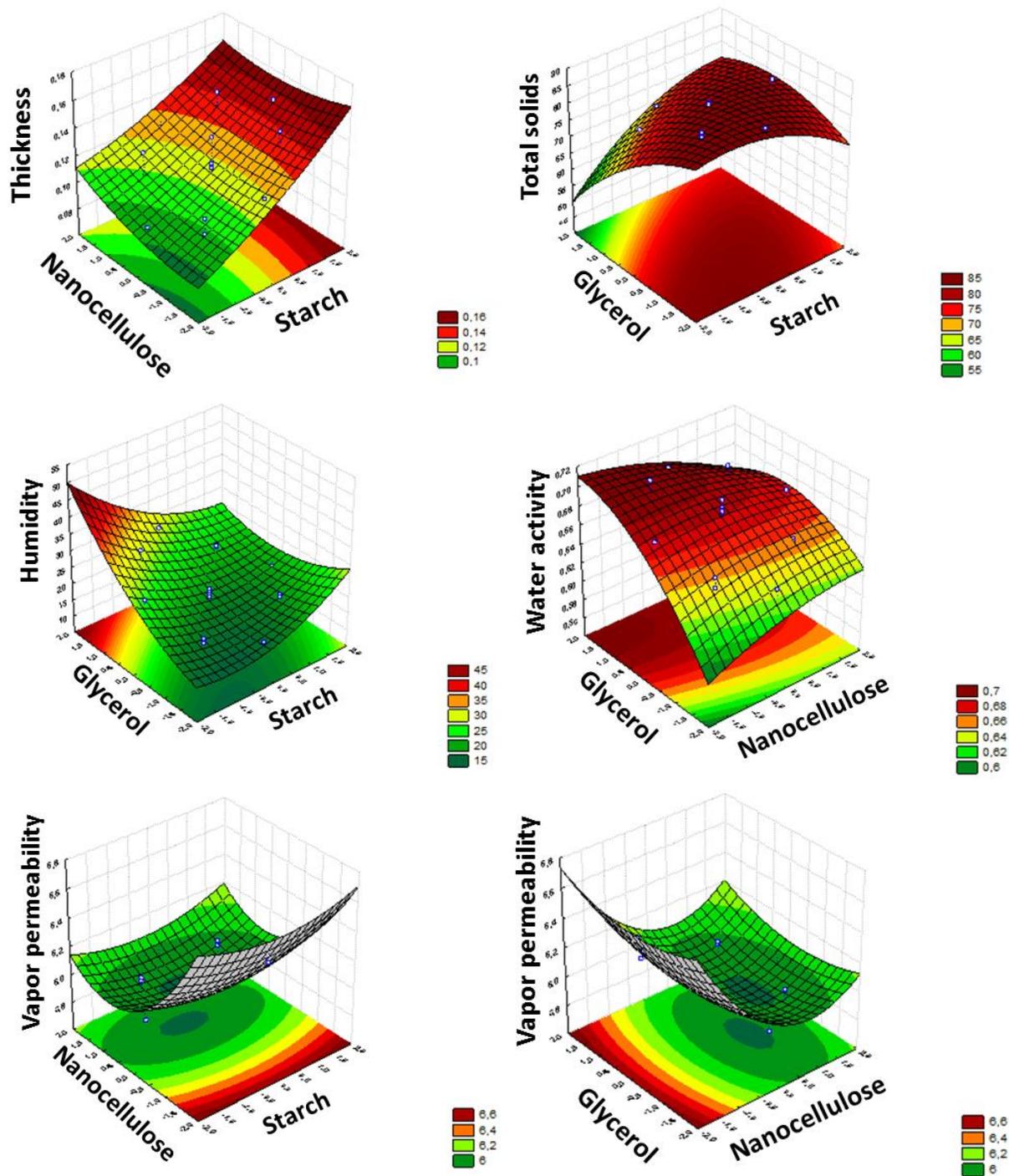


Figure 2. Response surfaces generated for the interactions of the independent variables (starch, glycerol and nanocellulose) on the dependent variables: thickness, total solids, humidity, water activity (aw) and water vapor permeability (PWV) of the 17 formulations of nano-biocomposites.

the resistance to tensile of the nanocomposites from 5.4 to 12.7 MPa (135%), the Young's modulus from 0.5 to 1.8 MPa (252%), and the rupture tension from 35.8 to 84.6 MPa (136%), compared with the control. Montero et al.

(2017) reported that the incorporation of cellulose nanoparticles favoured plasticization and increased the rigidity in thermoplastic starches films and moisture resistance. Azeredo et al. (2009) prepared edible films

Table 4. Equations of the model and R^2 (determination coefficient) for thickness (T, mm), total solids (TS, %), humidity (H, %), Water Activity (aw), Permeability to water vapor (PWV, gH₂O/m.s.Pa) of the films, X_1 = Cassava starch, X_2 = Glycerol and X_3 = Coconut nanocellulose.

Parameters (Independent variables versus Independent variables)	Equation	R^2
Thickness (X_1 versus X_3)	$0.117 + 0.015X_1 + 0.014X_1^2 + 0.024X_3 + 0.001X_3^2 - 0.001X_1X_3$	0.97
Total Solids (X_1 versus X_2)	$81.62 + 1.458X_1 - 1.474X_1^2 - 3.834X_2 - 1.359X_2^2 - 2.387X_1X_2$	0.97
Humidity (X_1 versus X_2)	$18.37 - 1.458X_1 + 1.474X_1^2 + 3.834X_2 + 1.359X_2^2 - 2.387X_1X_2$	0.97
Water activity (X_2 versus X_3)	$0.682 + 0.018X_3 - 0.006X_3^2 - 0.019X_2 - 0.002X_2^2 - 0.006X_2X_3$	0.97
PWV (X_1 versus X_3)	$5.939 + 0.004X_1 + 0.029X_1^2 - 0.138X_3 + 0.096X_3^2 - 0.003X_1X_3$	0.98
PWV (X_2 versus X_3)	$5.939 + 0.012X_3 + 0.029X_3^2 - 0.138X_2 + 0.096X_2^2 + 0.003X_2X_3$	0.98

Table 5. Average values (\pm sd – standard deviation) of the dependent variables regarding the mechanic properties of the nano-biocomposites and control (C) and percentage of alterations in relation to control.

F	E (MPa)	\uparrow E (%)	σ (MPa)	\uparrow σ (%)	ϵ (%)	\downarrow ϵ (%)
Control	1.00 \pm 0.24	-	0.87 \pm 0.12 ^f	-	100.44 \pm 4.05	-
F1	26.71 \pm 1.41	2.670	2.81 \pm 0.11	323	71.72 \pm 1.01	28.6
F2	29.19 \pm 0.97	2.919	3.01 \pm 0.32	346	68.38 \pm 1.75	31.9
F3	4.56 \pm 0.32 ^a	456	0.91 \pm 0.07 ^{a,f}	105	29.00 \pm 0.89	71.1
F4	5.89 \pm 0.83	589	1.03 \pm 0.10 ^{a,c}	118	27.91 \pm 1.30	72.2
F5	13.25 \pm 1.52 ^b	1.325	1.89 \pm 0.21 ^{b,d}	217	59.02 \pm 1.17	41.2
F6	55.25 \pm 7.20	5.525	3.82 \pm 0.36	439	14.91 \pm 1.11	85.15
F7	10.98 \pm 0.95	1.098	1.59 \pm 0.08	183	40.00 \pm 1.01	60.2
F8	15.09 \pm 1.06 ^c	1.509	1.91 \pm 0.15 ^{b,d}	219	38.36 \pm 0.91	61.8
F9	4.81 \pm 0.65 ^{a,d}	481	1.01 \pm 0.11 ^{a,c}	116	40.86 \pm 1.13	59.3
F10	34.19 \pm 1.13	3.419	3.22 \pm 0.41	370	56.18 \pm 1.20	55.9
F11	470.90 \pm 9.07	47.090	14.09 \pm 1.22	1.619	6.01 \pm 0.43	94.0
F12	4.89 \pm 0.43 ^a	489	1.06 \pm 0.03 ^c	122	89.02 \pm 1.54	11.4
F13	13.93 \pm 0.90 ^b	1.393	1.81 \pm 0.89 ^d	208	37.54 \pm 1.21	62.6
F14	18.98 \pm 1.21	1.898	4.02 \pm 0.18	462	37.98 \pm 0.87	62.2
F15*	15.04 \pm 0.76 ^{c,d}	1.504	2.45 \pm 0.35 ^e	281	50.29 \pm 1.09 ^a	50.1
F16*	14.94 \pm 1.45 ^{c,d}	1.494	2.05 \pm 0.64 ^e	277	49.16 \pm 0.98 ^a	51.0
F17*	15.13 \pm 0.82 ^d	1.513	2.42 \pm 0.65 ^e	278	50.36 \pm 1.22 ^a	49.9

Formulations (F); *Central Points. \uparrow or \downarrow : increase in relation to control. Values that show the same letter, in the same column, do not have significant differences ($p > 0.05$) by the Tukey Test at 95% confidence level. E, Young Module (MPa); σ , maximum tension (MPa); ϵ , deformation (%).

from mango puree that were strengthened with nanofibers of commercial cellulose. They observed that the nanocrystals were efficient in increasing the resistance to tensile, and this effect on the Young's modulus was even more noticeable at higher concentrations, which suggests the formation of a fibrillar structure inside the matrix.

Cellulose nanocrystals obtained from any natural source, are responsible for improving the mechanic properties of films when incorporated into the films that are composed of either biodegradable or synthetic matrixes. However, in this study, the incorporation of nanocellulose could not be observed as an exclusive parameter because the formulations were prepared with

simultaneous variations of two other components, starch and glycerol. According to the Pareto graphs for tension and modulus (Figure 3), the plasticizer glycerol also played an important role in improving the mechanic properties of the studied nano-biocomposites. Glycerol is a small molecule that facilitates its insertion within the polymer chains, exerting a higher influence in their mechanic properties. The polar groups (-OH) of the plasticizer molecules incur plasticizer-polymer interactions in substitution of the polymer-polymer interactions in the polymeric mixtures. Additionally, the starch underwent a structural modification after a thermal treatment facilitated the interaction of glycerol with its chain and allowed a

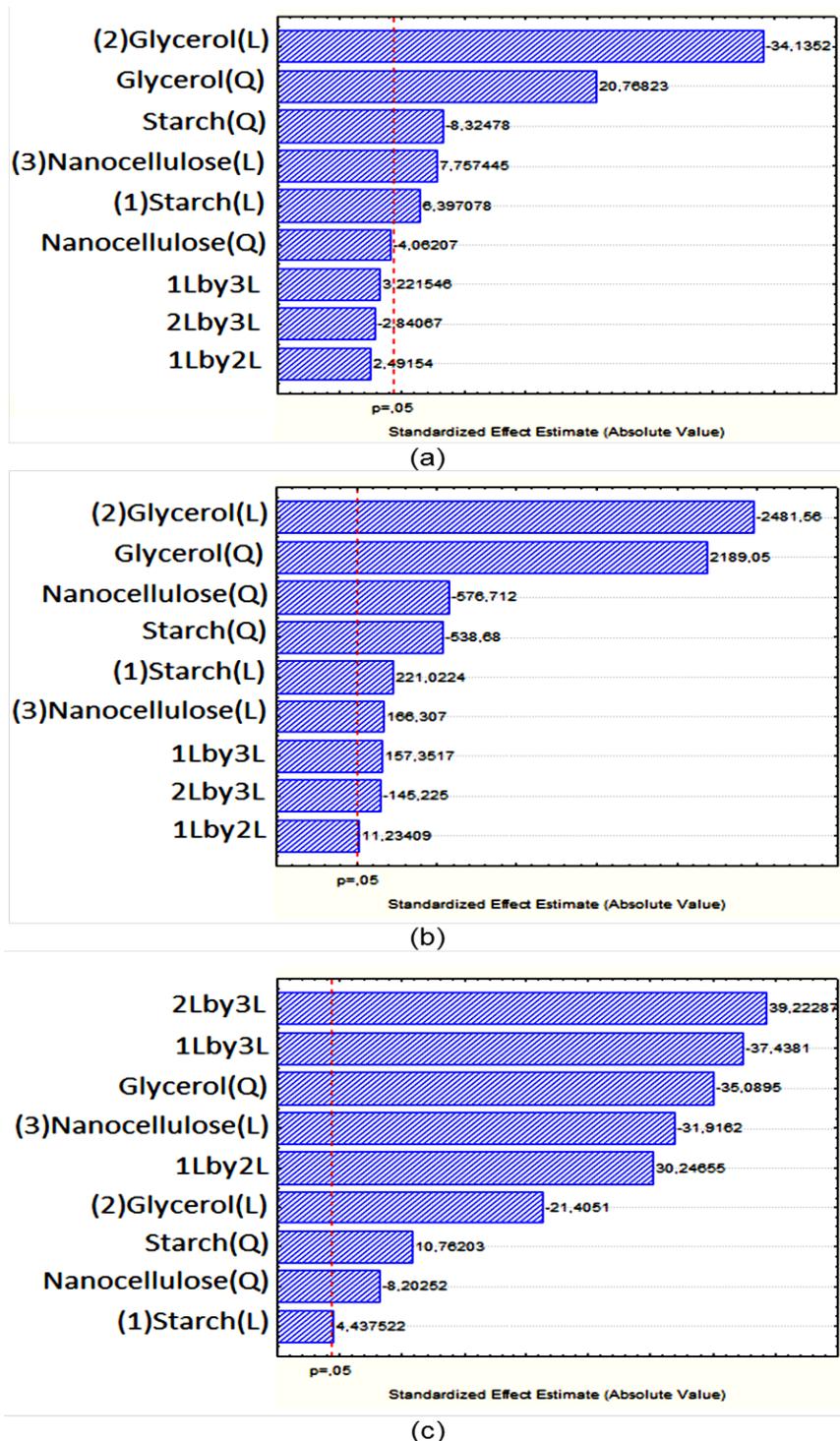


Figure 3. Pareto graphs for (a) module, (b) tension and (c) deformation of the nano-biocomposites.

greater interaction between plasticizer-starch-nanocellulose; thus, this contributed to improving the mechanic properties of the films. Therefore, the incorporation of nanocrystals modifies the mechanical

properties, despite the presence or absence of the plasticizer.

Generally, the plasticized films with higher glycerol concentrations are more flexible and have higher

elongation values, whereas the films with lower plasticizer content showed higher modulus and tension values. Glycerol is a hydrophilic plasticizer that interacts with the starch chains, increasing the molecular mobility and consequently the hydrophilicity and flexibility of the plasticized films (Mali et al., 2004). Formulation F11 showed the highest modulus and tension values, whereas F12 had the highest elongation value. These results showed the efficiency of glycerol as a plasticizer, which was also proved in studies for films manufactured with starch by Shimazu et al. (2007), with lactoglobulin by Sothornvit and Krochta (2001) and with soy protein by Cho and Rhee (2002). Azeredo et al. (2010) have demonstrated that lower concentrations of glycerol and higher concentrations of commercial cellulose nanocrystals provide an increase in the mechanic properties of the films obtained from chitosan. According to the Pareto graph (Figure 3), the concentrations of glycerol (X_2) and nanocellulose (X_3) exerted a higher effect on the maximum tension value, whereas the linear interactions between glycerol (X_2) and nanocellulose (X_3) (2Lby3L), and starch (X_1) and nanocellulose (X_3) (1Lby3L) were the primary factors responsible for the deformation percentage (elongation) of the nano-biocomposites.

The nano-biocomposites examined in this study can be considered complex systems that showed competitive interactions among all the present components in variable concentrations. The incorporation of nanocellulose from coconut was determined as efficient in decreasing the water permeability of the films and improving the mechanic properties of the system and thus widening the applications of nanocellulose from coconut materials. Finally, the isolation of cellulose nanocrystals constitutes an excellent alternative to the reutilization of ligno-cellulosic residues and their application as support additives in polymeric materials.

Conclusion

The results shown in this study demonstrate that it is possible to obtain nanocellulose from green coconut fibers through the process of acid hydrolysis (H_2SO_4 64%, 50°C, 10-15 min) with an L/D ratio of 38.9 ± 4.7 . The incorporation of these nanocrystals in cassava starch films plasticized with glycerol contributes to significantly improving the mechanical properties of films, such as Young's modulus and maximum tension. These effects consequently decrease the elongation percentage of the films. When compared with the controls, all formulations showed an increase in the Young's modulus. Formulation F11 reached the upper limit with an approximate 47.000% increase and a consequent decrease in elongation of 94% compared with the control. In addition, the presence of nanocrystals in the polymeric matrix of starch improved the barrier properties, such as water vapor permeability and water activity of the films.

Therefore, films formulated from cassava starch and

plasticized with glycerol can have their mechanic and barrier properties significantly altered by the incorporation of coconut cellulose.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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