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André Luiz Missio

NOVAS ESTRATÉGIAS PARA VALORIZAÇÃO DO EXTRATO DE TANINO

Santa Maria, RS,
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Tese apresentada ao programa de Pós-Graduação em Engenharia Florestal, área de concentração em Tecnologia de Produtos Florestais, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Engenharia Florestal**

Orientador: Prof^{o} Dr. Darci Alberto Gatto

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Santa Maria, RS
2017

Aos meus queridos e amados: pais, LUIZ E DALVA
esposa, NAYARA
e familiares...

... dedico esse trabalho.

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“O conhecimento serve para encantar as pessoas, não para humilhá-las.”

Dr. Mario Sergio Cortella

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Engenharia Florestal
Universidade Federal de Santa Maria

NOVAS ESTRATÉGIAS PARA VALORIZAÇÃO DO EXTRATO DE TANINO

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DATA E LOCAL DA DEFESA: SANTA MARIA, 21 DE JULHO DE 2017

Em um mundo em busca de produtos de baixo impacto ambiental, a correta exploração de recursos biológicos está se tornam-se uma necessidade. Taninos condensáveis estão presentes em diversas fontes na natureza e caracterizam-se por serem o segundo fenólico mais abundante da Terra, atrás somente da lignina. Industrialmente, os taninos condensados são utilizados, em sua maioria, no curtimento de couro, como adesivos e também floculantes. Com base na produção mundial desse extrato fenólico e o crescimento de pesquisas sobre maximização do aproveitamento de uma matéria prima, o presente trabalho teve por objetivo a elaboração de novas estratégias para valorização do extrato de tanino. Para tanto, tanino de Mimosa (*Acacia mearnsii* De Wild) foi utilizado inicialmente em um processo de purificação, a fim de eliminar açúcares e ácidos e assim obter novas frações do extrato. O tanino foi fracionado utilizando solventes orgânicos com diferentes polaridades em um extrato soxhlet, em processos simples e sequenciais. Posteriormente, a fim de aplicar o tanino industrial de Mimosa em produtos ou novos materiais, confeccionou-se compósitos plástico-madeira utilizando o tanino como um compatibilizante natural. Os compósitos foram preparados utilizando polipropileno, resíduos de pinus e fibras de madeira, em um processo com um homogeneizador termocinético e uma prensa hidráulica. Por fim, o tanino foi incorporado a nanocelulose para confecção de um filme com capacidade de aplicação em embalagens ativas. Celulose e o tanino foram misturados com água destilada em um microprocessador de fibrilação mecânica, formando um gel homogêneo com celulose nanofibrilada e tanino. Utilizando um sistema de filtração a vácuo, o gel foi transformado em um filme. Os principais resultados obtidos através de caracterizações específicas das frações, compósitos e filmes foram muito positivos. Diferentes propriedades foram observadas nas frações, sendo possível direcionar o material obtido para fins específicos, como para fármacos e embalagens ativas (menor peso molecular e maior capacidade antioxidante) e adesivos (maior peso molecular). Acréscimos no módulo de armazenamento e na hidrofobicidade foram obtidos a partir da incorporação do tanino em compósitos plástico-madeira, além de uma compatibilização ser confirmada por análises morfológicas. Por fim, os filmes de nanocelulose e tanino apresentaram elevada propriedade antioxidante, liberada a partir do contato com a água. Ainda, os filmes mostraram elevada resistência mecânica e capacidade de barreira, sendo indicados para embalagens ativas para alimentos e fármacos. Portanto, nós acreditamos que o extrato de tanino pode e deverá ser utilizado para elaboração de materiais com maior valor agregado, principalmente devido a sua estrutura química, abundância e ciclo sustentável de produção.

Palavras-chave: biorrefinaria, purificação, compósitos plástico-madeira, compatibilizante, embalagens ativas.

ABSTRACT

Doctoral Thesis
Forest Engineering Graduation Program
Federal University of Santa Maria

NEW STRATEGIES TOWARD THE VALORIZATION OF TANNIN EXTRACT

AUTHOR: ANDRÉ LUIZ MISSIO
ADVISOR: DR. DARCI ALBERTO GATTO
DATE AND PLACE OF DEFENSE: SANTA MARIA, JULY 21th, 2017

In a world looking for low environmental impact products, the correct use of each biological resource is needed. Condensable tannins are present in many natural sources and they are the second most abundant phenolic compound in the Earth, behind only of the lignin. Based on both the worldwide production of this phenolic extract and the increase in the researches to improve the use of raw materials, this doctoral thesis aimed to elaborate new strategies toward the valorization of the tannin extract. Mimosa tannin (*Acacia mearnsii* De Wild) was first purified to remove carbohydrates and acids to obtain new fractions of the extract. The tannin was fractionated using organic solvents with different polarities in a Soxhlet extraction system with single and sequential processes. After that, wood-plastic composites were made using tannin as a natural compatibilizer to apply the Mimosa industrial tannin into products or new materials. The composites were prepared with polypropylene, pine sawdust and wood fibers through a thermokinetic homogenizer and a hydraulic press. Finally, the tannin was incorporated into the nanocellulose to develop a film for application in active packaging. Cellulose and tannin were mixed with distilled water in a mechanical fibrillation microprocessor, forming a homogeneous gel with nanofibrillated cellulose and tannin. Using a vacuum filtration system, the gel was converted into a film. The main results obtained through specific characterizations of the fractions, composites and films were very positive. Different properties were observed in the fractions, and it was possible to target the material obtained for specific purposes, such as for pharmaceuticals and active packaging (lower molecular weight and higher antioxidant capacity) and adhesives (higher molecular weight). Increases in the storage modulus and in the hydrophobicity were obtained from the incorporation of the tannin into wood-plastic composites, besides a compatibilization to be confirmed by morphological analysis. Finally, the films of nanocellulose and tannin presented high antioxidant properties, which was released from the contact with water. Furthermore, the films showed high mechanical strength and barrier capacity, indicating their for application as active packaging for foods and drugs. Therefore, we believe that the tannin extract can and should be used for the elaboration of higher value-added materials, mainly due to its chemical structure, abundance and sustainable production cycle.

Keywords: biorefinery, purification, wood-plastic composites, compatibilizer, active packaging.

SUMÁRIO

1. ESTRUTURA DA TESE

As principais contribuições dessa tese estão relacionadas ao embasamento científico sobre taninos e posteriormente elaboração de novos materiais com características naturais, sustentáveis e a partir de uma fonte renovável. Nesse sentido, o trabalho foi estruturado em forma de artigos científicos, tendo como objetivo destacar os principais resultados e levar ao leitor facilidade de entendimento sobre o tema abordado.

O **Artigo 1** apresenta uma introdução sobre atuais e novos materiais baseados em extratos de tanino de Acácia Mimosa. Essa revisão foca em discutir os últimos e importantes avanços nesse campo de pesquisa. O artigo foi submetido a revista Maderas. Ciencia y tecnologia.

No **Artigo 2** nós reportamos um novo método de purificação de extratos de tanino. Nesse artigo, extrações sequenciais e simples com solventes orgânicos foram realizadas sobre o extrato de Mimosa, suas novas frações são comparadas e indicadas para determinados usos específicos. O artigo foi publicado na revista Separation and Purification Technology (Número da Licença 4130370256124).

A utilização do tanino de Acácia Mimosa como uma nova abordagem na compatibilização em compósitos plástico-madeira foi reportada no **Artigo 3**. Diversas análises foram realizadas para confirmação da hipótese inicial e a conclusão desse artigo se mostrou muito interessante no campo de pesquisas sobre madeira e derivados de madeira. Artigo publicado na revista Polymer Composites (Número da Licença 4155480687556).

No **Artigo 4** está apresentada a pesquisa destaque da tese. Nós preparamos filmes de nanocelulose e tanino utilizando uma nova técnica, com o objetivo de produzir embalagens "ativas" 100% naturais e sustentáveis a partir de componentes de árvores. As caracterizações desses filmes destacaram a elevada capacidade antioxidante quando o tanino foi adicionado, além de sugerir alta capacidade de barreira a líquidos. Nós acreditamos que esse novo "bio-nanocomposite" pode ser um material para embalagens no futuro no campo de alimentos e medicamentos. Artigo submetido a revista Journal of Cleaner Production.

Após a apresentação dos artigos, um capítulo de **Discussão** foi elaborado com o objetivo de unir as ideias descritas anteriormente, resultando em um fechamento das hipóteses lançadas durante os artigos. Finalmente um capítulo com as **Considerações Finais**, realçando os objetivos alcançados e sugerindo novas pesquisas nessa área.

2. MANUSCRIPT 1

EXPLOITING TANNIN EXTRACTS: INTRODUCTION TO NEW BIO-BASED MATERIALS

Explorando extratos de tanino: Introdução para novos materiais de base biológica

RESUMO

Em um mundo em busca de produtos de baixo impacto ambiental, a correta exploração de cada recurso biológico está se tornando uma necessidade. Com mais de 200.000 toneladas por ano, o extrato de tanino é o mais abundante fenólico extraído na Terra. Esse produto é constituído principalmente por moléculas polihidroxi-aromáticas, e já é industrialmente utilizado no processo de curtimento de couro, enologia e para clarificação de água. No entanto, devido à sua química de polimerização, os taninos condensados podem ser explorados também para outros fins, tais como adesivos e revestimentos. Nos últimos anos, polímeros baseados em taninos têm sido usados para síntese de espumas isolantes e preservativos de madeira, enquanto que por suas propriedades adesivas e antioxidantes foram incorporados com sucesso em compósitos plástico madeira (WPC) e filmes de celulose nanofibrilados, melhorando seu módulo de armazenamento e atividade antioxidante, respectivamente. A presente revisão introduz estes quatro materiais inovadores e algumas noções básicas para a purificação do extrato industrial Mimosa ou acácia negra (*Acacia mearnsii)*.

Palavras-chave: Materiais sustentáveis, proteção da madeira, espumas resistentes ao fogo, embalagem sustentável, nanomateriais, flavonoides.

Exploiting tannin extracts: Introduction to new bio-based materials

André Luiz Missio[a], Darci Alberto Gatto[a], Gianluca Tondi[b,c]*

Abstract: In a world seeking environmentally sustainable products, bio-resources are investigated as suitable replacements to oil-derived products. Tannin extracts represent one of the most abundant phenolic resources of the earth with more than 200,000 T/year. These extractives are comprised mainly of polyphenolic compounds and they are industrially used for various purposes, including leather tanning, wine-making, and water clarification. But the condensed tannin extractives can also be easily polymerized and the resulting bio-macromolecule can be exploited for other applications, such as adhesives and coatings. In recent years, tannin-based polymers have also been used for the synthesis of fire-resistant insulation foams and performing outdoor wood preservatives. This bio-resource imparts also outstanding water resistance to wood plastic composites (WPCs) and gives a great antioxidant activity to nanofibrillated cellulose films which become an interesting material for "active packaging". The literature review covers these four innovative solutions made from tannin extractives from mimosa or black wattle (Acacia mearnsii) industrial powder and gives also some basic information about the purification of the industrial tannin extract that can be suited for more chemically specific usages.

Keywords: Green materials; Timber protection; Natural foams; Sustainable packaging; Nanomaterials; Flavonoid fractions.

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INTRODUCTION

Environmental sustainability is currently a major focus in material science and bio-based resources are fundamental players for producing new materials for the future. Natural components having comparable performances to synthetic ones represent a major advantage in environmentally-friendly engineering.

In this context, the proper exploitation of various bio-resources will be a winning point for the future bio-economy that will rely on biorefineries. In these processes, bio-based feedstocks, such as agriculture and forest derived products will be transformed into bio-materials and bio-fuels (Reddy *et al.* 2010).

Wood usage is currently living a new golden age due to its unmatched properties. Not only because timber it is still one of the most reliable material for building construction purposes, but also because it is a CO_2 neutral and this renders this bio-resource even more attractive.

Accordingly, also the wood components are becoming always more valuable. The major components of wood, namely cellulose, hemicelluloses and lignin, are already used for a wide range of purposes (Carvalheiro *et al.* 2008, Hubbe *et al.* 2008, Moon *et al.* 2011, Ragauskas *et al.* 2014, Liu *et al.* 2016, Hubbe *et al.* 2017, Tabarsa *et al.* 2017). But nowadays, also the extractives representing between 1 and 10% of the dry wood (Umezawa 2000), are becoming an interesting feedstock because of their relatively easy supply (Schofield *et al.* 2001, Belgacem and Gandini 2008, Arbenz and Averous 2015).

Extractives are constituted of various molecules, such as low molecular mass sugars, terpenes and polyphenolics but the types and the relative proportions of the various extractives are strongly dependent to the wood species. For wood species like oak, chestnut, pine, quebracho, and mimosa, the most abundant chemicals are polyphenols and therefore the extracts are commonly called “tannins”. This name, indeed, refers to substances that are able to tan the leather (Haslam 1989). Therefore, the tannins are only the polyphenolic substances, while the hydrocolloids, sugars, and organic acids of the tannin extract are the “non-tannins” (Arbenz and Averous 2015). The two most common families of polyphenols in plant extracts are the hydrolysable and the condensed tannins. The former are esters of simple sugars with gallic or ellagic acid (Arbenz and Averous 2015), while the latter are oligomers or polymers of oligomeric flavonoids (proanthocyanidins), as shown in Figure 1 (Schofield *et al.* 2001, Hagerman 2002).

Tannins are the second most abundant phenolic resource in nature just behind the lignin. Condensed tannins (proanthocyanidins) constitute more than 90% of the total world production of commercial tannins (200.000 tons/year) and hence the condensed tannins are the most abundantly extracted natural substances on Earth (Jorge *et al.* 2001, Pizzi 2008).

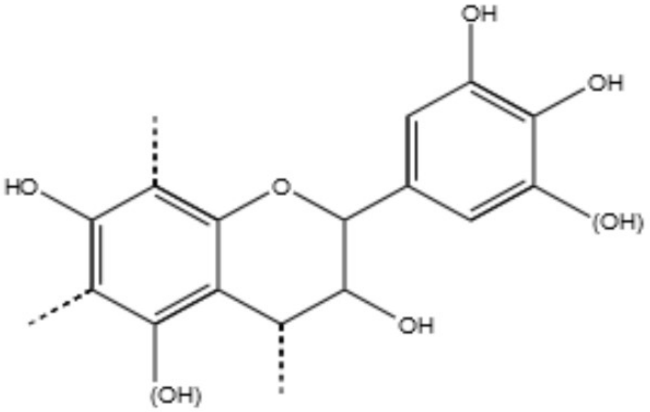

Figure 1. Chemical structure of a proanthocyanidin repeating unit of condensed tannin

Among the sources of condensed tannins, the industrial tannin extracts from mimosa (*Acacia mearnsii* De Wild) are the most sustainable because i) the bark contains from 30 to 45% of the tannins; ii) the tree species is fast growing with a plantation cycle of 7 years (TANAC 2017). Statistical data show that the surface of planted Acacia (mimosa) increased by 26% during the 2010 to 2015 period reaching 160.000 hectares in 2015 (IBA 2016). Regarding the economic use of the raw material, the value of the acacia bark is around \$90/T, while the wood (with a density of 650 kg/m^3) is sold at around \$30/T (AGEFLOR 2015, Delucis *et al.* 2016). Acacia mimosa wood is principally used for charcoal and pulp production. Mimosa tannin extracts are obtained by hot water extraction of bark chips in a counter-current series of autoclaves, using different parameters of temperature, pressure and time according to the required final properties of the extract (MIMOSA 2017, Missio *et al.* 2017a, c).

Condensed tannins have been used for centuries for leather tanning (Pizzi 2008), and for decades for the production of adhesives (Carvalho *et al.* 2014), wines (Rinaldi *et al.* 2016), and flocculants for water treatment (Beltrán Heredia and Sánchez Martín 2009). More recently, the use of these phenolic substances was also examined for their antioxidant and antifungal activities in pharmaceutical products (Wei *et al.* 2015, Aires *et al.* 2016, Missio *et al.* 2017c), for coatings (Pan *et al.* 2015), and for the synthesis of advanced ultra-lightweight materials (Amaral-Labat *et al.* 2013). In particular, polymers synthesized from mimosa extractives resulted in plastics that were less brittle than those made from other condensed tannins, and the polymerization of mimosa extractives was more easy to control (Pizzi 1994).

This literature review examines the application of the tannin extracts from Acacia mimosa for the synthesis of innovative materials, such as wood preservatives, insulation foams, wood plastic composites, and nanocellulose films. Finally, a short overview is also provided on the purification method for this phenolic bio-resource.

NEW BIO-BASED MATERIALS

Wood Preservation

Wood can be affected by extrinsic factor that limits its service life (Cademartori *et al.* 2015b, Missio *et al.* 2016). Wood quality decreases with outside exposure, and the rate of deterioration is dependent to the timber species (Lazarotto *et al.* 2016), the preservation technology applied and the environmental conditions (e.g., weather, soil, etc.). Among these factors, the preservation technology applied is the factor that can be more easily controlled to enhance the service life of wood (Magalhães *et al.* 2012). Over the years, many processes have been developed in order to protect wood against xylophagous agents (Cademartori *et al.* 2015a) and the severe environmental restrictions related to the use of heavy-metal based preservatives, like creosote, copper-chrome-arsenic (CCA) and more recently copper-chrome-boron (CCB) have renewed interest in finding more environmentally sustainable preservatives (Figure 2).

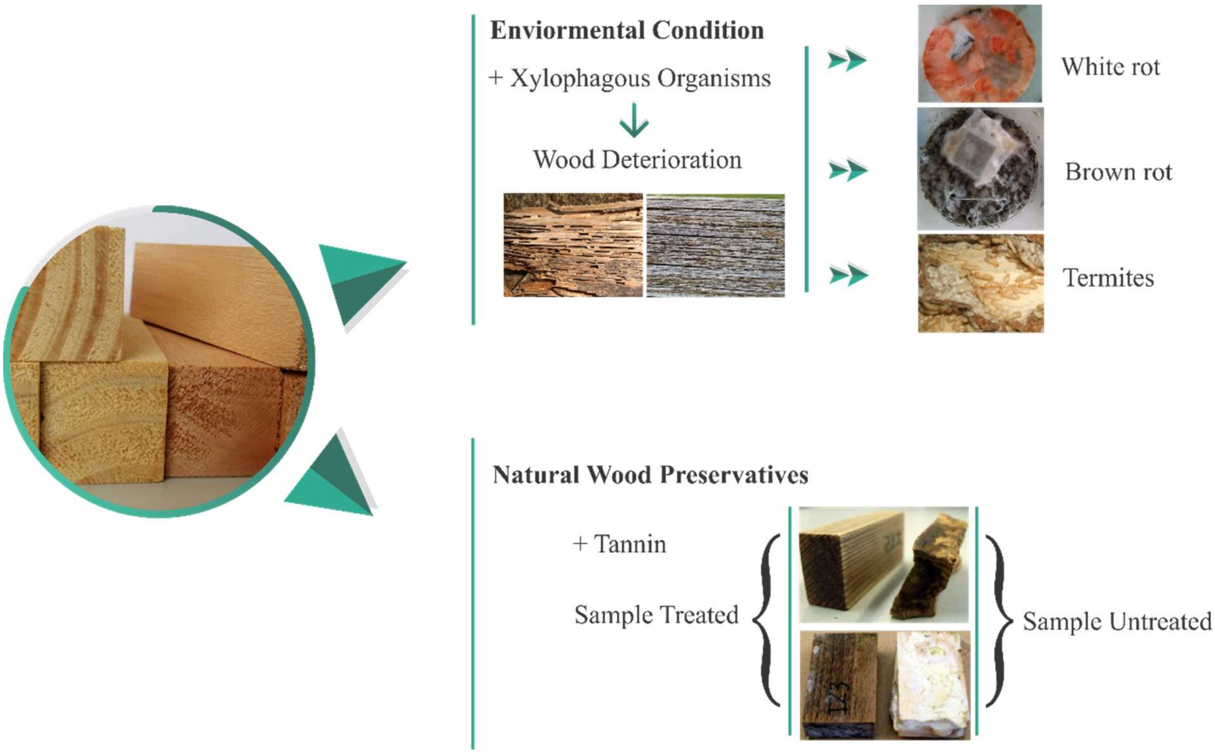

Figure 2. Wood deterioration with and without natural tannin preservative

According to Lebow (2010), wood preservatives must meet two broad criteria: i) they must provide the desired wood protection for the intended end use and ii) they must do so without presenting unreasonable risks to people or to the environment. Therefore, the idea of protecting wood with wood-derived preservatives has been studied over the past decades by several research groups.

In this context, tannin-based preservatives are a very attractive bio-mimetic solution. Increasing the concentration of these substances, already synthesized by the trees to protect themselves, helps to protect the wood against biologic and UV-light attacks during its service life as a building material (Hagerman *et al.* 1998, Tondi *et al.* 2013b).

Extractives obtained from mimosa, quebracho and pine have shown moderate resistances to biologic attack by fungi and termites (Tascioglu *et al.* 2013); this resistance was enhanced with the addition of copper and/or boron salts (Scalbert *et al.* 1998, Yamaguchi and Yoshino 2001, Yamaguchi *et al.* 2002). However, every study in which leaching processes were proposed, serious problems in relation to treated wood and water were observed (Tondi *et al.* 2012a, Tondi *et al.* 2013a). Logically, tannins which are obtained by water extraction results also highly soluble after application. A novel approach has been proposed to overcome the leaching problems that involves the *in-situ* polymerization of condensed tannins. Formulations containing hexamine as hardener have been proposed and such preservatives exhibited outstanding biologic resistance against *Pycnoporus sanguineus* (Thevenon *et al.* 2008). These initial findings obtained with water-based tannin formulation added of a contained amount of hexamine (6%) and boron (<1%) started a research line carrying to several interesting results: i) limited tannin and boron leaching due to the polymerization; ii) wide-spectrum biological resistances (against fungi and insects); iii) improved mechanical and fire properties (Tondi *et al.* 2012a, Tondi *et al.* 2012b, Tondi *et al.* 2013a, Tondi *et al.* 2013b).

Unfortunately, only moderate weathering resistance was observed. Indeed, the rigidity of the tannin polymers and the sensitivity against radical degradation strongly affect the outdoor application of this formulation (Tondi *et al.* 2013a). However, several studies have been performed and are still ongoing in order to increase the elasticity of the hardened polymers so that this drawback can be solved (Hu *et al.* 2017, Tondi *et al.* 2017).

Tannin Foams

Other interesting tannin-based materials are the tannin foams (Figure 3). These porous materials are obtained by copolymerization of the tannin extract with furfuryl alcohol in acid environment. The obtained copolymer cures simultaneously with the evaporation of a low-boiling point solvent resulting in a lightweight porous material (Tondi and Pizzi 2009). These tannin foams are black porous solids that have a skeletal structure and are completely bio-derived: condensed tannin extract represents the largest component (60-80%) while furfuryl alcohol, a derivate molecule of lignocellulosic biomass and hemicelluloses (Aguilar *et al.* 2002, Climent *et al.* 2014, Canhaci *et al.* 2017), is the remaining part (20-40%). In the tannin foams, different molecules can be added for improving some specific properties. Formaldehyde and isocyanates contribute for the increasing of the mechanical properties (Tondi *et al.* 2009, Li and Ragauskas 2012); polyurethanes increase the elasticity (Basso *et al.* 2014) while polyaniline can be used in order to obtain a semi-conductive material (Tondi *et al.* 2015).

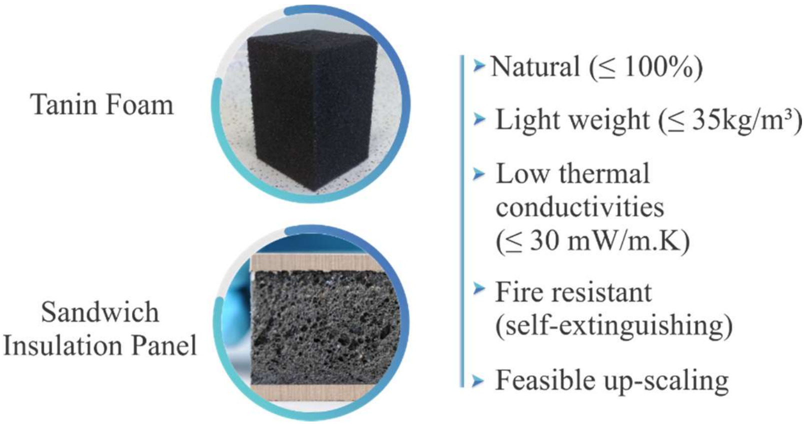

Figure 3. Tannin foam and sandwich insulation panels

The wide range of formulations developed in the last decade allows to considered the tannin foams for a large set of applications (Link *et al.* 2011, Kolbitsch *et al.* 2012, Tondi *et al.* 2014 , Tondi *et al.* 2015). In particular, the light tannin foams have shown low thermal conductivities and good fire resistances, which makes them an ideal insulation material. Hence, these foams have already been produced in a semi-industrial scale (Tondi *et al.* 2016b). Interesting perspectives are represented by the different processing technologies introduced in the last years. The tannin-furfuryl alcohol copolymer can be hardened at room temperature, but also with the external heat sources like conventional ovens (through convection), hot-presses (conduction) and microwave and IR radiations (Link *et al.* 2011, Kolbitsch *et al.* 2012, Tondi *et al.* 2014). These processing modifications further extend the applicability of the material because they contribute in shortening the production time. The selection of the proper production method can allow to reduce the amount of catalyst and hardener requested, finally leading to the production of a sustainable tannin foam tailored for specific applications.

Wood-Plastic Composites

Wood plastic composite (WPC) are materials containing wood-derived resources (e.g., fibers, sawdust, and wood flour) combined in a matrix of a thermoplastic polymer (e.g., polyethylene (PE) or polypropylene (PP)) (Figure 4). WPC are used in various building construction materials, such as flooring, exterior cladding and decking (Ashori *et al.* 2013). These construction materials combine the mechanical resistance of wood with the formability and hydrophobicity of the polyolefin while being environmentally sustainable.

Figure 4. Examples of raw material components in WPC products

The advantages of WPCs are that they are more environmentally sustainable than plastic alone, and they offer higher durability, lower maintenance and lower abrasion wearing than wood alone (El-Haggar and Kamel 2011).

Polymeric composites can be made using a matrix of high density polyethylene, HDPE (Zadorozhnyy *et al.* 2016), polyethylene glycol, PEG (Tsuboi *et al.* 2016), polyethylene terephthalate, PET (Merijs Meri *et al.* 2014), or polyvinyl chloride, PVC (Yazdani *et al.* 2016). However, the polypropylene, PP (Mattos *et al.* 2014, Cademartori *et al.* 2015c, Cademartori *et* *al.* 2017) is used more widely for applications like automotive components, electrical devices, food packaging and household equipment (Izzati Zulkifli *et al.* 2015). Its market leading position of the WPCs is due to the combination of economic (Thakur *et al.* 2014a, Thakur *et al.* 2014b) and technologically performing features (Ayrilmis *et al.* 2015).

Various lignocellulosic resources, like sugar cane fibers, ramie, jute, flax, pineapple, sisal, coconut fiber, castor seed cake, cotton, pupunha cover and wood residues, have been used to derive fibers and flours used in composite plastics (Satyanarayana *et al.* 2009, Magalhães *et al.* 2013, Mattos *et al.* 2014, Cademartori *et al.* 2015c). Nevertheless, the market is largely dominated by WPCs where the lignocellulosic resource is wood (Wood-Plastic 2017).

The main technological drawback for producing WPCs is the limited adhesion affinity between the wood and the plastic matrix components. Substances that “bridge” these two materials are called compatibilizers and their use represent for this material the principal scientific frontier. Until now, one of the most known WPC compatibilizer is maleic anhydride (Tufan *et al.* 2015) which contributes in increasing the mechanical strength of the composite. Recently, renewable substances, like lignin, have been used successfully as a WPC compatibilizer that increases the thermal stability and the storage modulus of the resulting composites (Lee *et al.* 2015). These positive results encouraged the investigation of other bio-resources as compatibilizers, like tannins, which are smaller molecular structures when compared to lignin but retain similar functional groups and structure. Furthermore, this bio-resource present less variability than lignin carrying to a is more consistent and reliable compatibilizer.

These studies have highlighted that the mimosa tannins plays an active role in filling the gap at the interfaces between PP and wood and this compatibilization was confirmed by the enhanced storage modulus and hydrophobicity of the WPCs surface (Missio *et al.* 2017a).

Nanocellulose-Tannin Films

Thin films of wraps to bags are extensively used in packaging for the protection of delivered goods from dirt, germ, liquid or gas contaminations (McKeen 2013). These films are usually prepared from polypropylene (PP), low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE) since these plastics are readily available, low cost, light weight and inert. In Europe, 49 millions of Tons/year of oil-derived plastics are produced, of which almost 40% is used for packaging purposes (Plastic 2017). This trend is no longer sustainable and the need for more natural alternative materials with similar properties is growing exponentially (de Léis *et al.* 2017, Wang and Wang 2017).

Simultaneously, the packaging industry is beginning a "new age" in which the packaging being inert and impermeable is not always sufficient. Delivered goods may require also some chemical support (such as antioxidant and microbiological properties) during the storage phase and therefore "active packaging" was created (EU 2009). These materials interact with the goods to be protected by releasing antioxidant or antimicrobial compounds into the items to enhance preservation (Al-Naamani *et al.* 2016, De Vietro *et al.* 2017), resulting in prolonged shelf-life.

Future trends indicate that a new frontier of research in this field is the synthesis of active packaging using environmentally sustainable resources. Interestingly, trees provide the basic chemical components to produce active package for the future: cellulose and tannin (Figure 5) (Missio *et al.* 2017b).

Figure 5. Films formed from nanocellulose and tannin.

Cellulose is the most abundant biopolymer on the earth, its production estimated at about 10^{11} tons per year. Cellulose can be found in nature in two forms. The first form is call of pure cellulose and is present in cotton, some algae cellulose, and bacterial cellulose. The second form is call cellulose complex, which is present in most plants found in nature as a fundamental component in the cell wall (Pecoraro *et al.* 2008).

Inserted in the environmental scenario and the sustainability of new product development, cellulose and, more specifically, fibrillated or nanofibrillated (or cellulose nanofibers – CNFs) can play an extraordinary role (Valle-Delgado *et al.* 2016, Hubbe *et al.* 2017). CNFs have dimensions of approximately 5-60 nm in diameter and a length of a few micrometers; these nanofibers are produced through a mechanical fibrillation (friction), and in some cases with the aid of chemical or enzymatic pre-treatments, of cellulosic pulps (Iwamoto *et al.* 2008, Isogai *et al.* 2011, Klemm *et al.* 2011). One of the main attributes of CNFs, which adds to their value, is the high mechanical strength, such as the high modulus of elasticity that ranges between 10 to 150 GPa (Iwamoto *et al.* 2009, Lee *et al.* 2012).

The production of nanocellulose films is similar of nano-papers (Urruzola *et al.* 2014). Nanofibrils suspended in water generate a gel that after filtration, produce a very dense film (Sehaqui *et al.* 2010). This formed structure shows interesting combination of high modulus of elasticity, tensile strength and barrier properties (water and/or gas diffusion). This makes such films attractive for industrial applications like bio-based packaging (Moon *et al.* 2011, Lavoine *et al.* 2015, Lavoine *et al.* 2016, Hubbe *et al.* 2017).

When tannin is embedded within the nanofibrillated cellulose, the resulting film showed highly antioxidant activity when in contact with water and this may also represent a certain protection against fungi (Yamaguchi *et al.* 2002, Pizzi *et al.* 2004, Tondi *et al.* 2013b, Missio *et al.* 2017c). It was observed that nanocellulose films impregnated with tannin results in a film surface that is more hydrophobic than nanocellulose only films, which is due to an intimate interconnection between the flavonoid tannin and the cellulose (Missio *et al.* 2017b). Such a new, naturally derived material represents one of the most promising “active packaging” with antioxidant properties (Olejar *et al.* 2014, Zhou *et al.* 2016).

Tannin Purification and Fractionation

The four innovative materials mentioned in the previous sections have the great advantage of using industrially-available material. However, the presence of non-tannins in the raw tannin powder limits the application of this bio-resource when more controllable applications are requested. Hence, purification of the industrial extract is required when the tannin has to be utilized for more advanced purposes (Figure 6).

The tannin industrial raw material containing “non-tannins” can be produced and used in large volumes for low value product (e.g. leather tannin, water treatments). Conversely, after purification lower yield of higher added value products are also possible for specific purposes (e.g. films/packages, foams, compatibilizers and antioxidants) rendering the purification process economical viable (Luong *et al.* 2012). In addition, diversifying the use of raw materials and processing technologies, as well as reducing dependence on the production of only one product, can provide new combinations needed in different market areas (Ghatak 2011). For instance, secondary metabolites, such as gums, terpene resins and tannins that are derived from forest resources, can be used for the production of high value-added chemicals, such as cosmetics, pharmaceuticals, animal feeds and food flavours (Naik *et al.* 2010).

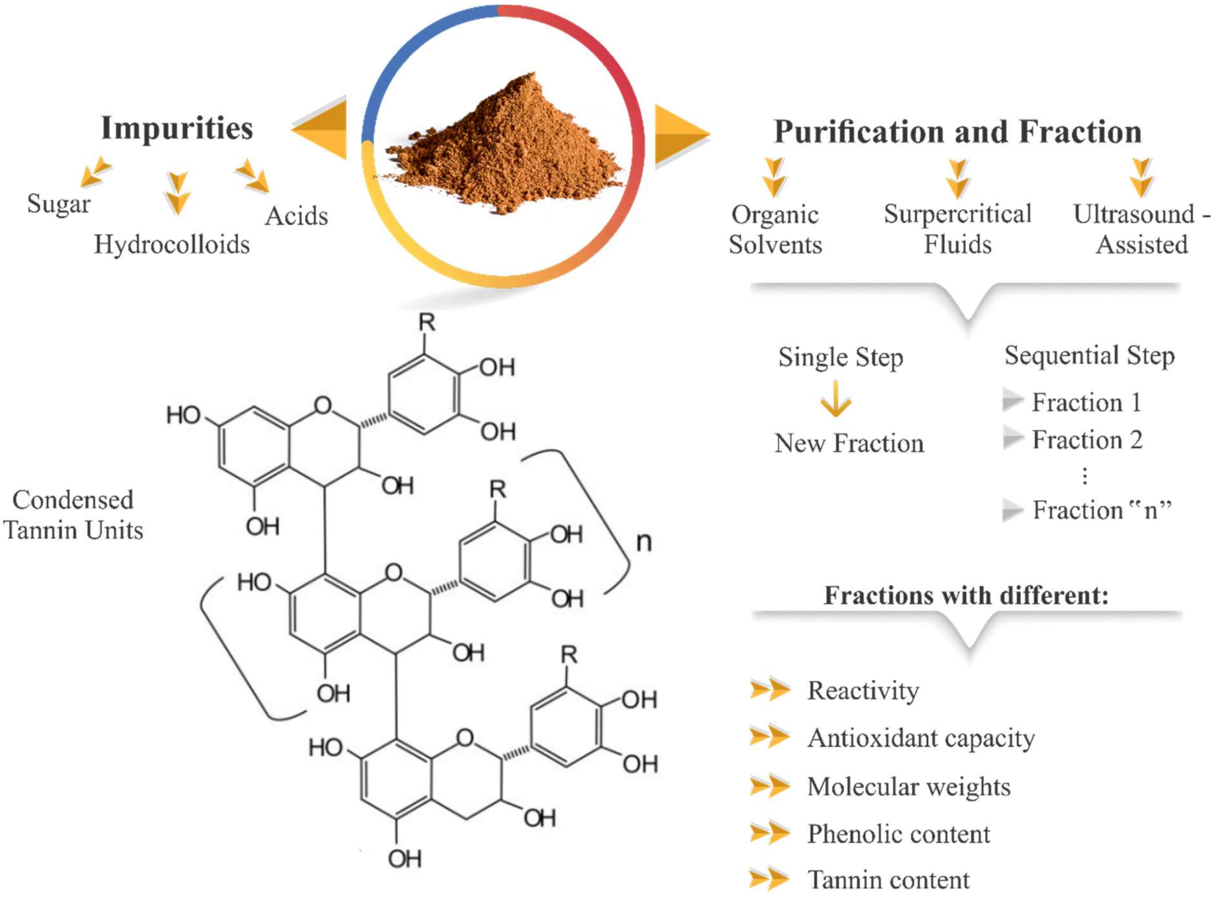

Figure 6. Various tannin fractionation methods and resulting purified fractions

The separation of wood extractives has a long history. The main process of tannin fractionation is countercurrent chromatography (Putman and Butler 1985). The chromatography process usually uses a Sephadex LH-20 column (Tibe *et al.* 2013), or newer techniques, such as supercritical fluid and ultrasound assisted extraction (Pansera *et al.* 2004, Sousa *et al.* 2014). These methods required complex analytical techniques and expensive equipment; hence, easier methods for the fractionation have been developed. In fact, very few studies have been published regarding this topic (Teng *et al.* 2013, Teng *et al.* 2015) even though the soxhlet method has been shown to efficiently fractionate other hydroxy-aromatic compounds such as lignin (Yuan *et al.* 2009, Li and McDonald 2014). Hence, black liquor can be decontaminated and fractionated to yield phenolic monomers of high commercial value (Erdocia *et al.* 2015) be used as biofuels (Gordobil *et al.* 2016), rigid foams (Li and Ragauskas 2012, Xue *et al.* 2014, Tondi *et al.* 2016a) and composite additives (Gordobil *et al.* 2015, Spiridon *et al.* 2015).

In general, fractionation using organic solvents is characterized by successive extractions with different solvent polarities in order to purify the raw material into fractions containing specific molecular weights components with specific characteristics. Accordingly, fractions with different molecular weights, antioxidant capacities, condensed tannins, ash levels and phenolic contents can be obtained, each of which can be used for targeted purposes (Missio *et al.* 2017c). Specific tannin fractions can also be isolated through sequential solvent extractions when particular chemical compositions are required.

CONCLUSIONS

Tannin is a very interesting bio-resource for plenty of applications and recently several new bio-based materials containing considerable amounts of tannin were successfully produced.

i) Timber preservatives based on tannins have shown very high biological and water resistance and are already suitable for indoor wood preservation.

ii) Insulation foams derived from tannin and furfuryl alcohol present low densities, low thermal conductivities and high fire resistances resulting in an alternative product to synthetic commercial foams, such as polystyrene and polyurethane.

iii) Wood and polypropylene can be compatibilized with tannin in WPCs increasing the storage modulus and surface hydrophobicity.

iv) Films of nanofibrillated cellulose fortified with tannin have shown higher hydrophobicity and antioxidant activity producing a new composite with strong potential as active packaging material for food and pharmaceutical goods.

The purification of the industrial tannin extracts by targeted fractionation processes allows to produce fractions which can be exploited for improving the properties of the already produced tannin-base material of for the synthesis of chemically controlled materials such as ordered aerogels, xerogels or slow chemically releasing structures.

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3. MANUSCRIPT 2

ANALYTICAL CHARACTERIZATION OF PURIFIED MIMOSA (*Acacia mearnsii*) INDUSTRIAL TANNIN EXTRACT: SINGLE AND SEQUENTIAL FRACTIONATION

Caracterização analítica de extratos de taninos purificados de Mimosa (*Acacia mearnsii*): Fracionamento simples e sequencial

RESUMO

Extrato de tanino de Mimosa (Acácia negra) é uma das poucas fontes industriais naturais de polifenóis. Mesmo se a composição desses extratos é bem conhecida, atualmente poucas tentativas de purificação foram realizadas. Assim, utilizando método de extração Soxhlet com diferentes solventes, frações foram obtidas e investigadas analiticamente. Rendimento, massa molecular, teor de fenólicos e taninos, atividade antioxidante e espectroscopia FT-IR mostraram que a fração obtida com acetato de etila contém elevada capacidade antioxidante, taninos de baixo peso molecular (também hidrolisáveis), enquanto que as frações alcoólicas contêm flavonoides purificados com alto peso molecular e, os seus resíduos são enriquecidos de carboidratos e cinzas. O fracionamento sequencial otimizou a classe de componentes extraídos de acordo com a aplicação específica. Foram observadas significativas correlações entre fenólicos, atividade antioxidante, massa molecular e cinzas.

Palavras-chave: GPC, Folin-Ciolcateu, ensaio vanilina, capacidade antioxidante, ORAC, hidrocolóides, extração sólido-líquido.

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Analytical characterization of purified mimosa (*Acacia mearnsii*) industrial tannin extract: Single and sequential fractionation

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ABSTRACT

Mimosa (Black wattle) tannin extract is one of the few industrially available natural sources of polyphenols. Even if its composition is rather known, limited attempts for its purification were done until now. A Soxhlet extraction, using various solvents, with single and sequential processes was performed, and the separated fractions were analytically investigated. Yield, molecular mass, phenolic and condensed tannin contents, antioxidant activity and FT-IR spectroscopy have shown that the ethyl acetate fraction strongly contains antioxidants and low molecular mass tannins (also hydrolyzable), while the alcoholic fractions contain purified flavonoids with higher molecular mass; the final residues resulted enriched in carbohydrates and ashes. Sequential extractions optimize the class of compounds extracted according to the specific application. Conforming to statistical analysis, significant correlations have been observed between phenolic content and the antioxidant activity, as well as between molecular masses and ashes content.

1. Introduction

In a world where alternative and more sustainable resources than crude-oil are required, plants derivatives are the principal feedstock for bio-refineries and green chemistry processes [1,2]. In this context, the plant extracts are a very promising opportunity for supplying phenolic substances [3]. For some species, such as chestnut, oak (for hydrolyzable tannins), mimosa and quebracho (for condensed tannins), the extracts are particularly rich in polyhydroxyphenols. The high yields found in the extracts justify their industrial distillation [4].

Tannins have been used for centuries for leather tanning [4–6] and decades for the production of adhesives [7,8], oenology [9], as well as flocculants for water treatment [10]. More recently, the use of these phenolic substances was introduced also for pharmaceutical and advanced material purposes [4,11–14].

With about 200 thousand tons/year, corresponding to around 90% of the worldwide production, the condensed tannins are the most abundantly extracted natural substances [4]. This particular family of tannins, known as polyflavonoids and proanthocyanidins [5,15], is very interesting because they couple their strong antioxidant and antifungal activities [16] with a good chemical reactivity towards aldehydes, protein and other reagents [4]. Due to the latter feature, the condensed tannins can be considered as a suitable bio-resource for the synthesis of adhesives [17], coatings [18], wood preservatives [19,20] and even porous materials like foams [13,14,21] and aerogels [22]. The industrial extract of Acacia mimosa (*Acacia mearnsii*) is the most convenient for the synthesis of new materials because (i) it is the most sustainable – the Acacia mimosa is a fast growing tree, abundantly available, and its bark contains up to 45% of tannins [23] – and (ii) it is more performing: the mimosa-based polymers are less brittle and more easily manipulated than the other condensed tannins [7].

However, the mimosa extract is not completely constituted of phenolic substances [14]: easy sugars, organic acids and hydrocolloids are also contained in the extract [24]. A purification process is

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therefore required to refine the chemical composition, especially when applied for advanced purposes.

The concept of purification of biomasses is the same being developed in the bio-refineries to obtain "green" chemicals [25]: it starts from easily available bio-resources in order to retrieve pure chemicals or specific families of chemicals. The actual market trend indicates an increasing interest for the use of natural & natural-derived materials for several applications, like food antioxidant and pharmaceuticals [26–29]. Indeed, the tannin extracts can be still used in large volumes for leather tanning and flocculation purposes; however, purified substances can be applied for higher added value purposes, such as pharmaceutical, food and cosmetic, as well as for more technologically advanced materials, such as thin films, wood-plastic composites, foams and aerogels [13].

Currently, the main tannin fractionation and purification methods use countercurrent chromatography (CCC) [30], usually with the use of Sephadex LH-20 [31,32]. These methods require complex analytic techniques and expensive equipment, therefore, an easier approach for the fractionation could be considered. Not many studies have been presented on this subject [33,34], although the "Soxhlet" method has already been proven to efficiently purify other hydroxy-aromatic organic compounds, such as lignin [35].

In this context, the present study proposes a fractionation and purification method of tannin industrial extracts applying different organic solvents. Antioxidant activities, total phenolic and condensed tannins contents, as well as the fractions molecular masses, were measured to observe the properties of the extract and to evaluate if this purification method could be considered for specific applications. Single and sequential extractions with hexane, ethyl acetate, pentanol, propanol and methanol were applied in order to purify tannin industrial extracts.

2. Materials and methods

2.1. Raw material

The tannin industrial extract was kindly supplied by SETA® industry, located in the town of Estancia Velha – RS (Brazil). The extract was obtained from the bark of the *Acacia mearnsii* – black wattle through an industrial process. The extraction of tannin was obtained by a simple water extraction with specific temperatures (75-85 °C), pressure and time, in a series of autoclaves performing a countercurrent process. The extracted liquor was concentrated and air-dried to fine powder before packing. According to the industry data, the tannin extract is composed of condensed tannins, around 70–80%, and 20–30% in hydrocolloid gums, sugars and small molecules.

The solvents hexane, ethyl acetate, 1-pentanol, 1-propanol and methanol were all analytical grade and purchased by Sigma-Aldrich, Brazil.

The reactants were: Folin-Ciocalteau reactant (Merck, Germany), HCl (Diadema, Brazil), dimethylformamide (DMF), dimethylsulphoxide (DMSO), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, gallic acid and catechin (Sigma–Aldrich, USA), monobasic and dibasic potassium phosphate and sodium carbonate (Vetec, Brazil) and vanillin (Nuclear, Brazil).

2.2. Solvent fractionation

For each purification, 15 g of raw tannin industrial extract (M_T) were separated with five organic solvents with different relative polarity (Rp) and boiling temperature (Bt): Hexane; ethyl acetate; 1-pentanol; 1-propanol and methanol. The single fractionation process was carried out directly on the industrial extract, producing a soluble fraction called extract and an insoluble fraction called residue. Conversely, the sequential fractionation was carried out using the same solvents following the polarity sequence: hexane, ethyl acetate, 1-pentanol, 1-propanol and methanol, according to Fig. 1. In this technique, the residues of the previous separation were purified with the next solvent (e.g. the insoluble part of the hexane extraction was separated in ethyl acetate, and so on). Six replicates were performed for each purification.

The extractions were performed in a Soxhlet extractor for 6 hours at the boiling temperature of each solvent. The extracted fractions and the residues were both dried in an oven at 70 °C and the weight of the remained fraction (M_F) was registered.

The extract yield (EY) was calculated as in Eq. (1).

$$\mathrm{EY} = \left(\frac{M_F}{M_T}\right) \cdot 100 \qquad (1)$$

M_F = final mass after purification (g); M_T = initial mass of tannin extract (g).

2.3. Gel permeation chromatography (GPC)

The solid tannin fractions were dissolved in a dimethylformamide (DMF) forming a 3% (w/w) solution. They were left under magnetic stirring for 24 hours to ensure complete dissolution. The solution was then filtered and the filtrate was injected into the chromatograph. Size exclusion chromatography analysis were used to evaluate the average molecular weight (Mw) and number average molecular mass (Mn) of the fractions. N,N-Dimethylformamide (DMF) eluent was used as GPC mobile phase, at 35 °C and a flow rate of 0.7 mL/min, using a Jasco Inc. chromatograph provided with an LCNetII/ACD interface, a column oven CO-2065Plus and a RI-2031Plus Intelligent Refractive Index Detector. A guard column and two columns PolarGel-M (Varian Inc.) were employed. Calibration was made using polystyrene standards provided by Fluka, ranging from 250 to 70,000 Da [36,37].

2.4. Total phenolic content

The total phenolic content (TPC) was measured following a modified Folin-Ciocalteau method (Chandra and Mejia 2004). Briefly, 0.25 mL of 4 N Folin-Ciocalteau reagent were added to 1 mL of each sample (0.05 mg/mL); this mixture was kept reacting for 5 min before the addition of 2 mL of 20% sodium carbonate. The solution was stored for 10 min before measuring the absorption at 730 nm using an UV–visible spectrophotometer Agilent 8453 (Agilent Technologies, USA). Gallic acid was used as standard and the samples were measured in triplicate. The total phenolic content was expressed in milligram equivalents of gallic acid per gram of extract (mgGAE/g). For the quantification, it was used the equation obtained with the standard gallic acid (0.005–0.04 mg/mL): $y = 34.9368x - 0.0008$ ($r = 0.9999$). This analysis was performed in triplicate.

2.5. Total condensed tannin

Total condensed tannin content (TCT) was determined by the vanillin method, according to Morrison et al. [38]. In this methodology, to each sample at the concentration of 50 mg/mL (0.1 mL) were added methanol (0.9 mL), solution A (8.0 mL of concentrated HCl in 100.0 mL methanol, 2.5 mL) and solution B (1.0 g of vanillin in 100.0 mL methanol, 2.5 mL). After immersion in a water bath for 20 min., the absorbance at 500 nm was registered with the UV-spectrometer. Catechin was used to obtain the calibration curve

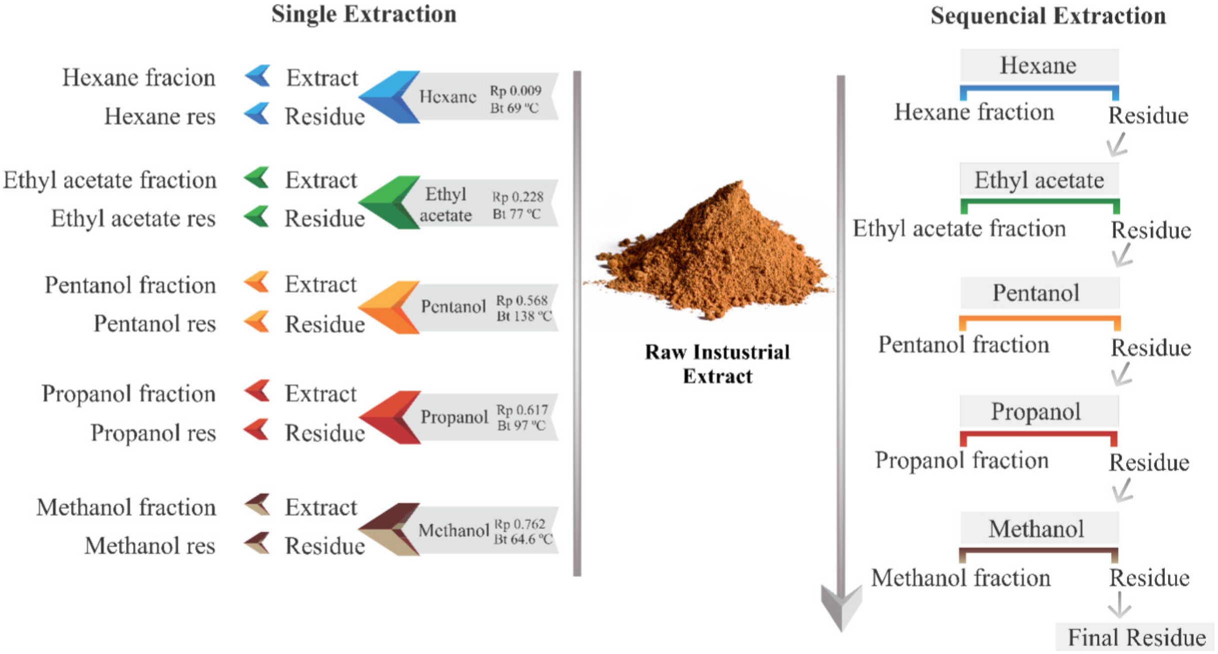

Fig. 1. Diagram of single and sequential extractions of Mimosa tannin raw industrial extract.

and the tests were performed in triplicate. The condensed tannins content was expressed in milligram equivalents of catechin per gram of extract (mgCE/g). For the quantification, it was used the equation obtained with the standard (5–60 mg/mL of catechin): $y = 0.025x + 0.173$, $r = 0.9935$. This analysis was performed in triplicate.

2.6. Antioxidant activity of tannin fractions

The antioxidant activities of all the tannin fractions were determined using the oxygen radical absorbance capacity (ORAC) method, described by Ou et al. [39]. The ORAC assay is used to measure the ability of an antioxidant to protect the disodium fluorescein from oxidation catalyzed by peroxyl radicals [40]. This kinetic assay is based on the measurement of radical scavenging activity of extracts against peroxyl radicals, produced by the addition of the AAPH (2,2′-azobis-2-methylpropanimidamide, dihydrochloride) radical inductor. For the determination of the optimal extract concentration for analyze, preliminary tests were made with known concentrations ranging from 5 to 300 mg L^{-1} (diluted in ethanol); the optimal concentration was determined to be 10 mg L^{-1}. A volume of 25 μL of sample (10 mg L^{-1}) or Trolox solution was added to a potassium phosphate buffer 75 mmol L^{-1} (pH 7.4) on a microplate, with incubation for 10 min at 37 °C. 150 μL of disodium fluorescein solution (81 nmol L^{-1}) were used as indicator and 25 μL of AAPH (152 mmol L^{-1}) were added as peroxyl radical generator. The fluorescence was then measured every minute (emission and excitation wavelengths were 530 ± 25 and 485 ± 20 nm, respectively) with SpectraMax M5 (Molecular Devices, Sunnyvale, CA, EUA) at 37 °C for 90 min. The ORAC values were calculated by a regression equation obtained with Trolox solutions (0–96 μmol L^{-1}) and area under curve (AUC) of the fluorescein decay. This analysis was performed in triplicate and were expressed as μmol of Trolox equivalents (TE) per g of extract in dry weight. The AUC was calculated according to Eq. (2):

$$AUC = 1 + \frac{f1}{f0} + \frac{f2}{f0} + \frac{f3}{f0} + \cdots \frac{fn}{f0} \quad (2)$$

fn = fluorescence in a read cycle (1 min); f0 = fluorescence at zero time.

2.7. Ash content of tannin fractions

Ash content of each fractions were measured following the methodology described in TAPPI standard (T 211 om-02) [41]. This analysis was performed in triplicate.

2.8. ATR FT-IR analysis

Vibrational spectroscopic measurements were performed directly in the ATR device by laying the different dried powders in an intimate contact with the surface of the diamond. The samples were scanned with a Nicolet Nexus 470 spectrometer in the spectral range between 4000 and 600 cm^{-1} with resolution of 4 cm^{-1}. Triplicates of the 32 scans for powder measurements were run and averaged. The spectra were, then, baseline corrected and area normalized and finally the fingerprints region between 1800 and 600 cm^{-1} of the spectra were compared as already described elsewhere [13].

2.9. Data analysis

Pearson correlation (simple correlation analysis) was applied to calculate the relation between variables. Significance levels were defined at $p < 0.05$, $p < 0.01$ and $p < 0.001$. From the correlation results, the regression models of the variables of interest were adjusted as a function of coefficient of determination (r^2) and mean absolute error (MAE). Moreover, a p-value of 0.001 (corresponding to 0.1% of significance) and Durbin-Watson statistic ($p > 0.05$ – no indication of a series residual autocorrelation) was considered.

3. Results and discussion

3.1. Extraction yield

Two major parameters have to be considered when the extraction process is evaluated: the polarity of the solvent and the extraction temperature. Tannins can be considered relatively polar compounds, therefore, it would be expected that solvents with high polarities and high boiling temperature extracts the majority of the material. In Fig. 2 the extraction yields for single and sequential extractions are summarized.

For the single extractions, it can be observed that propanol and methanol are the two solvents which extract the higher amount of material (86.9% and 82.2% respectively). Far lower yields are registered for the less polar extracts: pentanol purifies 28.2% of the original material, ethyl acetate extracts only 6.1%, while hexane separates only traces (0.2%). For this reason, the extract by hexane will not be considered any further. When sequential extraction is applied, the original material is more homogeneously distributed between the different alcohol fractions. Pentanol extracts 30.1%, propanol 22.4% and methanol 30.9%, so more than 80% of the original material is collected in these fractions. Assuming that the yield of the single extraction of the polar solvent should register values comparable with the sum of the sequential extraction up to that solvent, the highest difference is observed for propanol, where the yield of the single extraction (86.9%) is around 20% higher than the sum of the sequential extracts of hexane (0.2%), ethyl acetate (6.0%), pentanol (30.1%) and propanol (22.4%). This difference can be due to extract modifications occurring during the heat-drying cycles.

3.2. Molecular mass distribution

The GPC analysis performed on the extracted fractions have shown that the molecular masses of the purified substances depend on the solvent used. In Table 1, Mw, Mn and the polydispersity index (Mw/Mn) for single and sequential extractions are reported.

Single and sequential extractions present similar results in terms of extracted molecular mass. The ethyl acetate fraction resulted to be the one having far lower molecular mass, while the alcoholic fractions present decreasing molecular masses with polarity increase. The higher molecular mass (Mw and Mn) polymers are purified when the extraction temperature is higher. In the sequential extraction, the average molecular masses for the alcoholic extracts result slightly higher because a significant amount of low molecular mass substances were previously "selectively" separated by ethyl acetate. The fractions with lower molecular masses have also lower polydispersity, which suggests that the bigger macromolecules are strongly included in the high molecular fractions (e.g. pentanol and propanol); on the other hand, smaller molecules can also be found in the fraction with higher average molecular mass, suggesting a higher selectivity for bigger molecules. The polydispersity index increase slightly for the sequential extraction, which means that the sequential extracts produce a broader distribution of molecular masses.

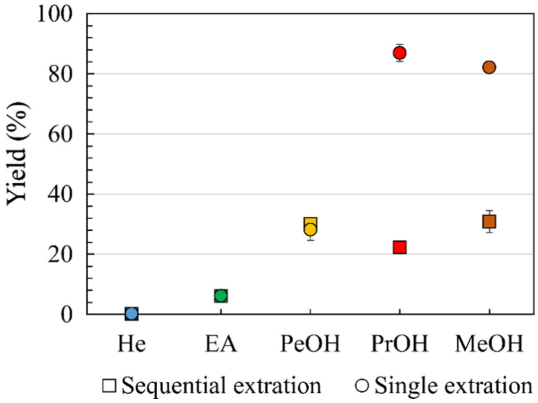

Fig. 2. Extraction yield of the single and sequential Mimosa tannin extractions.

Table 1
Mw, Mn and Polydispersity (Mw/Mn) of the tannin fractions for single and sequential extraction.

	Mw	Mn	Mw/Mn
Single extraction			
Ethyl acetate	1478	648	2.28
Pentanol	4234	990	4.28
Propanol	3677	939	3.92
Methanol	2146	741	2.94
Sequential extraction			
Ethyl acetate	1575	652	2.41
Pentanol	4734	1040	4.55
Propanol	3840	970	3.95
Methanol	3281	957	3.42

3.3. Total phenolic content (TPC) and total condensed tannin (TCT)

The determination of phenolic and condensed tannin content is fundamental to estimate the chemical properties of the fractions. In Fig. 3a and b the measurement of the two parameters are observed for the single and sequential extractions, respectively.

In the single extraction, it can be observed that TCP and TCT have completely opposite trends. Phenolic content increase with the polarity of the alcohols, while condensed tannins decrease; moreover, the ethyl acetate extract presents very high amount of phenolics and very low amount of condensed tannin. Being that condensed tannins are phenolics, it would be expected that the condensed tannin (TCT) and the phenolic (TPC) content correlate, but for analytical reasons this does not occur. The Folin-Ciolcateu method is relatively high selective for phenols [42], but the vanillin-method for condensed tannins presents two significant drawbacks: i) the fractions with lower molecular masses produce lower color intensity in the adduct with vanillin and ii) the lower reactivity of condensed tannin with resorcinolic A-ring reduce the response of these compounds. This means that the mimosa tannin extract, which is mainly robinetinidin (hence a 5-deossi proantocyanidin) results less sensitive to the vanillin assay [7,43]. In particular, we can state that the ethyl acetate fraction is the one containing lower molecular mass substances, while the pentanol fraction is the one which present the extract with higher molecular mass. According to this interpretation, in the sequential extraction we observe that the pentanol extract contains significantly less TCT than expected. This can be explained by the presence of higher molecular masses chains, which reduce significantly their reactivity against vanillin when exposed to sequential temperature/solvent stresses.

3.4. Antioxidant activity

The extracts were tested for their capacity to stabilize radicals and the results of the ORAC tests are reported in Fig. 4a for the single and Fig. 4b for the sequential extractions.

In both graphics it appears immediately that the antioxidant activity of the extracts is elevated and always higher than Trolox, a synthetic homologue of Vitamin E, which is suited as biological antioxidant for the high capacity of capturing ROS (reactive oxygen species) [44]. The extracts of the single extraction have a higher total antioxidant effect than the sequentially obtained extracts. This means that the low molecular mass extracts of ethyl acetate have a higher antioxidant effect than the other (total antioxidant activity, AUC = 4982 μmol TE/g). The lower antioxidant effect registered for the pentanolic fraction (total antioxidant activity,

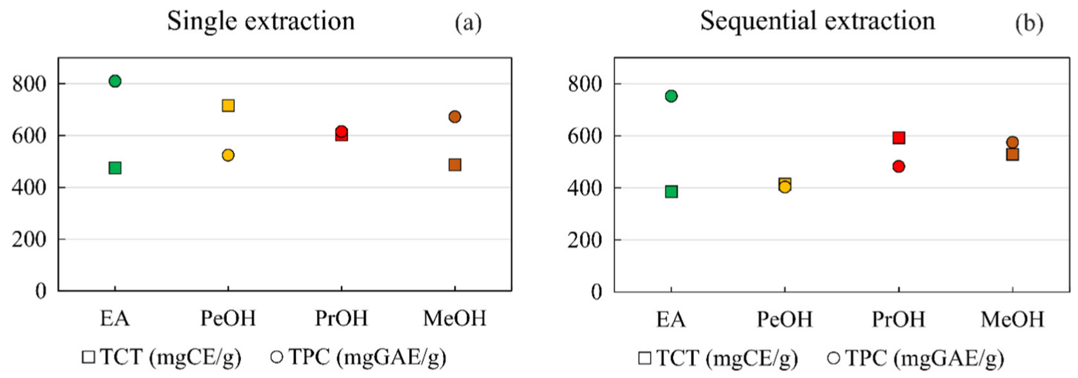

Fig. 3. Total phenolic (TPC) and condensed tannin (TCT) content for the single and sequential extracts.

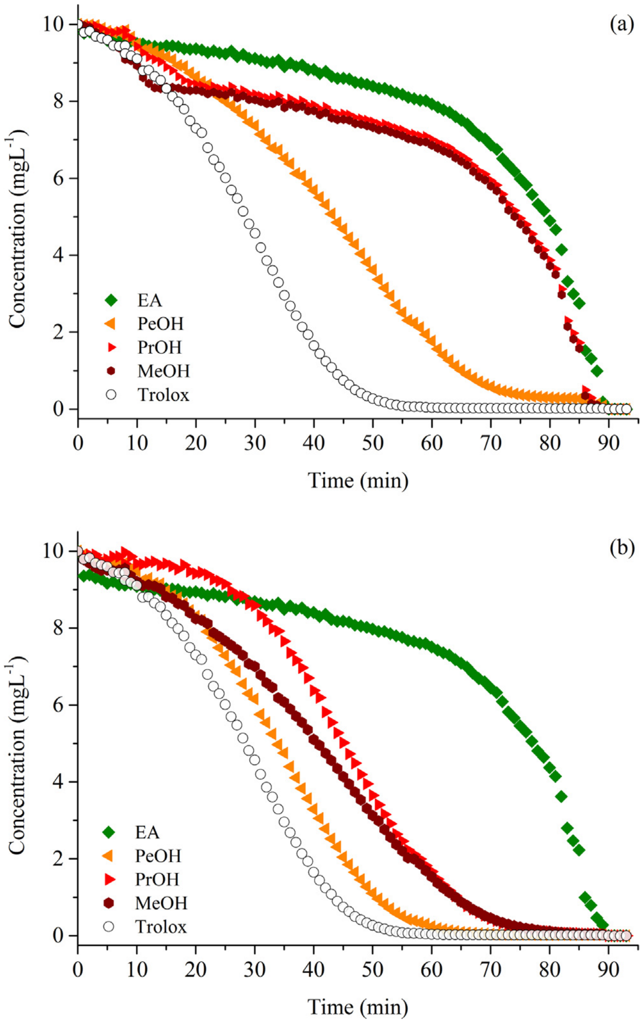

Fig. 4. Antioxidant capacity during time of the different tannin extract (a) single and (b) sequentially extracted in comparison with Trolox.

AUC = 3189 μmol TE/g) suggests that part of the hydrocolloids is solubilized in this fraction.

3.5. Residual fractions

The residual fractions were also analyzed and their properties are resumed in Table 2. The residual fractions present very different properties, which strongly depend on the extraction yield. It is interesting to observe that the residues with lower yields (higher extract yield), namely propanol, methanol and sequential residues, present: high molecular mass, high polydispersity index, low phenolic and condensed tannin content, low antioxidant activity and very high ash content. This means that high molecular mass carbohydrates and inorganic molecules accumulate in these fractions.

3.6. FT-IR analysis

More chemical information were obtained by fractions infrared spectroscopy, as shown in Fig. 5. The profile of the single extracted powders was found very similar for the alcoholic fractions, while the ethyl acetate fraction presented different peaks and intensities. In particular, the latter shows two new peaks at (i) 1705 cm^{-1} due to the carboxyl groups and (ii) 1260 cm^{-1} due to C—O stretching of the carboxyl group [45]; moreover, increased intensity were observed at 1510 cm^{-1} (C═C aromatic skeletal vibration), 1285 cm^{-1} (C—O stretching of pyrogallic ring), 1200 cm^{-1} (OH—plane deformation), 1100 cm^{-1} (CH bending phenolic in-plane) and 805 cm^{-1} (CH aromatic bending out-of-plane). On the other hand, the band at 1070 cm^{-1} (C—C bending of the B ring) decreases [46]. This information suggest that a small part (20–40%) of the ethyl acetate extract, which is only 6.1% of the entire extract, is constituted of hydrolizable tannins.

Much less difference can be observed between the alcoholic extracts: the spectra of the methanol extract present lower intensities at 1285 cm^{-1} and slightly higher absorption at 1100 cm^{-1}. These evidences would suggest that the methanolic fraction contains slightly less pyrogallic B-ring (more fisetinidin) and higher freedom for the C—H aromatic bendings, which could be explained also with a lower molecular mass. Pentanol and propanol extracts are almost the same, the only small difference in the spectra is found at around 890 cm^{-1}, where the pentanolic extract has a higher peak than the propanolic. This signal can be attributed to anomeric carbons in carbohydrates [47]. The spectrum of the total residue presents very high distance from the spectra of the extracted substances. New peaks can be observed at 1740 and 1150 cm^{-1}, which can be assigned to C═O stretching and C—O—C asymmetric vibrations. The signal at 1740 cm^{-1} suggests the presence of esters (hydroxyaromatic acid with carbohydrates), while the peak at 1150 cm^{-1} is typical for di- to poly-saccharides. The very broad bands between 1450–1350 cm^{-1} and 1150–950 cm^{-1} have to be principally attributed to carbohydrates [48,49]: the broadness suggest their polymeric arrangement. These spectral patterns suggest that the insoluble residues are carbohydrates which can be also covalently combined with some hydroxyl-aromatic compound.

Table 2
Summary of the results for the residues found in single and sequential extractions.

	Yield (%)	Mw (Da)	Mn (Da)	Mw/Mn	TPC (mgGAE/g)	TCT (mgCE/g)	ORAC (μmol TE/g)	Ashes (%)
Single extraction								
He	99.86 ± 0.08	2922	816	3.58	405 ± 11	161 ± 4	512 ± 18	5.10 ± 0.001
EA	94.86 ± 2.12	3306	945	3.50	320 ± 9	115 ± 12	454 ± 22	5.05 ± 0.02
PeOH	71.81 ± 2.97	3846	1169	3.29	380 ± 4	325 ± 1	1379 ± 19	7.9 ± 0.45
PrOH	13.03 ± 1.59	5743	1351	4.25	243 ± 7	107 ± 7	318 ± 6	14.5 ± 0.065
MeOH	17.83 ± 2.19	7063	1312	5.38	225 ± 11	6 ± 4	194 ± 6	12 ± 0.09
Sequential extraction								
Final residue	10.47 ± 1.70	4132	913	3.94	167 ± 8	204	6.7 ± 0.7	13.8 ± 0.07

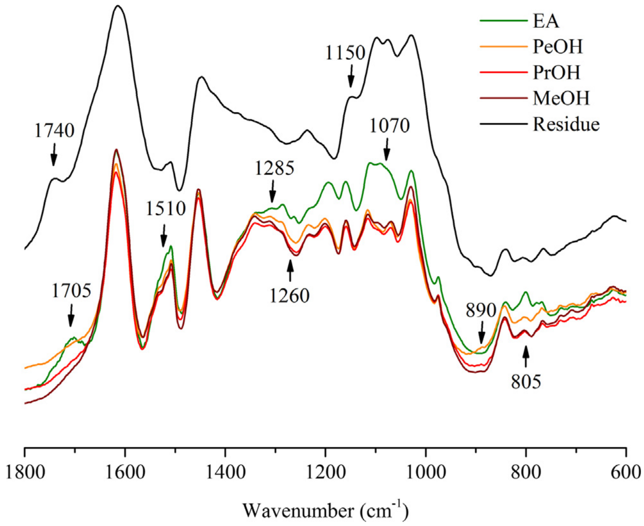

Fig. 5. FT-IR spectra of different tannin extracts and total residue.

3.7. Correlations

It is interesting to observe which variable correlate to others. In Table 3 the correlations are resumed.

The more significant correlations were observed for the phenolic content (TPC): particularly, extremely significant correlation occurred with the antioxidant activity, mass average molar mass and ashes content. The most important correlations highlighted through the Pearson analysis were further considered to estimate the regression models (Fig. 6).

This means that the Folin-Ciocalteau reaction on tannin extract can indirectly give very interesting information about the antioxidant activity and molecular mass of the polyflavonoid. These findings confirm the studies of Mokrani and Madani [50] and Price [43]. Furthermore, it is very interesting to see that the simple measurement of the ashes allows estimating (with a certain error) very important chemical features of the extract such as phenolic content, antioxidant capacity and molecular mass. The values of the coefficient of determination (r^2) and the significance (p value) of the regression models are reported in Table 3 for the most interesting Pearson correlations previously observed.

4. Conclusions

The purification of the industrial extract of Mimosa was performed. It was observed that the Soxhlet extraction of the tannin powders has higher yield for polar solvents and for higher extraction temperatures. In particular, the propanolic extraction is the one which guarantees yields of around 87%. The ethyl acetate extract resulted richer in polyphenols with low molecular mass and very high antioxidant capacity. A small amount of hydrolysable tannin, 1–2% of the total extract, was also observed. The pentanolic extract is constituted of high molecular condensed tannins, where a small portion of carbohydrates is also observed. The propanolic and methanolic fractions differ only for the slightly lower molecular mass of the methanolic fraction, which contain slightly higher portion of fisetinidin. The residues are enriched of carbohydrates/hydrocolloids and ashes content. These findings suggest that the extraction with different organic solvents was satisfactory, increasing the performance and application spectrum of the extracts/those new materials. Lighter fractions can be more easily suited as antioxidant, while heavier fractions can be ideal as adhesive filler (requiring less amount of hardener to cure).

The technique of sequential extractions has shown interesting applications to purify the fractions and in particular, the pre-extraction with ethyl acetate ensure the removal of the hydrolizable tannins. Extremely significant correlations have been observed between phenolic content and i) antioxidant capacity and ii) mass average molecular mass.

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Table 3
Pearson correlation coefficients between different assays (N = 13).

	Yield (%)	Mw (Da)	Mn (Da)	TPC (mgGAE/g)	TCT (mgCE/g)	ORAC (μmol TE/g)	Ashes (%)
Yield (%)	1						
Mw (Da)	−0.15[ns]	1					
Mn (Da)	−0.02[ns]	0.93[***]	1				
TPC (mgGAE/g)	0.03[ns]	−0.81[***]	−0.78[**]	1			
TCT (mgCE/g)	0.05[ns]	−0.48[ns]	−0.51[ns]	0.70[**]	1		
ORAC (μmol TE/g)	0.04[ns]	−0.77[**]	−0.79[**]	0.97[***]	0.75[**]	1	
Ashes (%)	−0.15[ns]	0.60[*]	0.65[*]	−0.78[**]	−0.74[**]	−0.78[**]	1

[ns] $p > 0.05$, no significant correlation

[*] $p < 0.05$, significant correlation.

[**] $p < 0.01$, very significant correlation.

[***] $p < 0.001$, extremely significant correlation.

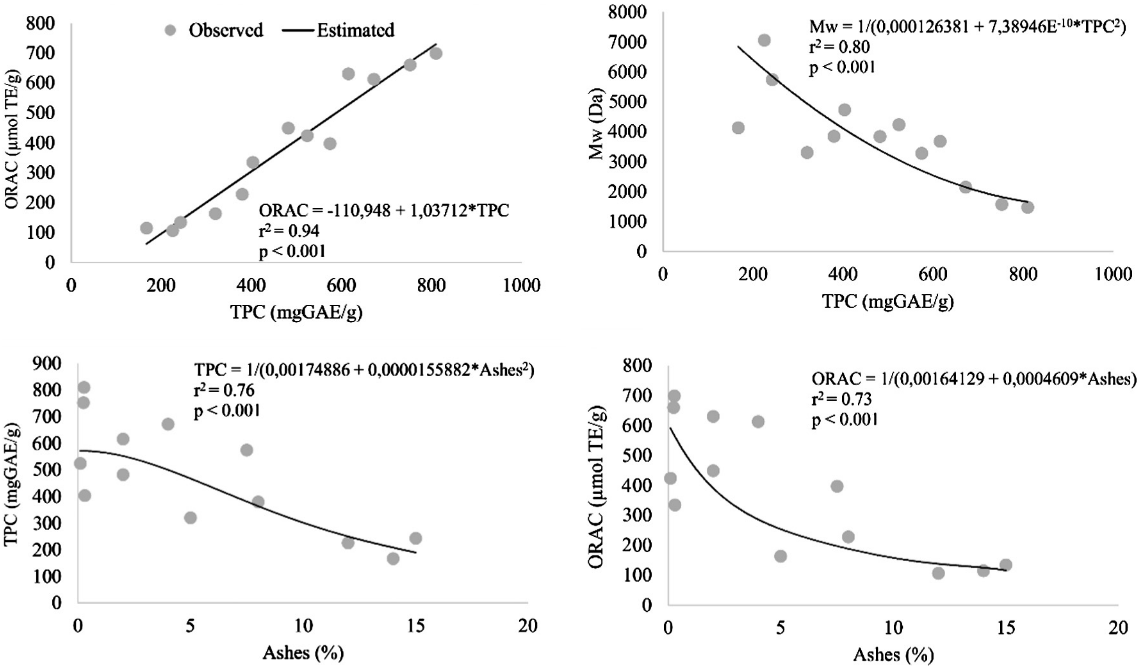

Fig. 6. Regression models between chemical properties of the different tannin extracts and residues.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2017.06.010.

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4. MANUSCRIPT 3

TANNIN AS SUSTAINABLE COMPATIBILIZER FOR WOOD-POLYPROPYLENE COMPOSITES

Tanino como um compatibilizante sustentável para compósitos madeira-polipropileno.

RESUMO

Compósitos plástico-madeira (WPCs) são produtos bem conhecidos que combinam as elevadas propriedades mecânicas da madeira com a hidrofobicidade e conformação do polipropileno (PP). WPCs são amplamente utilizados como decking, revestimento e pisos em construções ao ar livre, mas esse material ainda apresenta alguns inconvenientes devido a limitada afinidade entre as duas matérias primas. No presente trabalho foi testada a adição de tanino de Mimosa (*Acacia Mearnsii*) como compatibilizante e, estes compósitos, foram caracterizados via MEV, TGA, DSC, DMA, ensaios mecânicos, absorção de água e ângulo de contato. Com base nos resultados, o WPC com adição de tanino apresentou menores espaçamentos entre PP e a matriz lignocelulósica, maior hidrofobicidade e desempenhos mecânicos comparáveis. Nós acreditamos que esses resultados semelhantes foram devidos ao efeito da compatibilização que o tanino permitiu obter mesmo com 15% menos de material lignocelulósico. A menor cristalinidade observada para os compósitos contendo tanino permitiu um melhor contato entre as frações de madeira. Embora obtida uma boa compatibilização física, não foram observadas evidências de ligações químicas por FT-IR. Maiores ângulos de contato e hidrofobicidade registrada para os WPCs contendo tanino sugerem que os flavonoides podem ser aditivos adequados para melhorar a vida útil desse material em aplicações ao ar livre.

Palavras-chave: Compósitos madeira-plástico, compatibilizante natural, polifenóis, outdoor WPC.

Impact of Tannin as Sustainable Compatibilizer for Wood-Polypropylene Composites

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Wood Plastic Composites (WPCs) are well-known products which combine the high mechanical properties of wood with the high hydrophobicity and formability of polypropylene (PP). They are largely used for decking, cladding and flooring for outdoor constructions but they still present some drawbacks due to the limited chemical affinity of the two materials. In this paper, the addition of mimosa (*Acacia mearnsii*) tannin as compatibilizer was tested and these composites were characterized via SEM, TGA, DSC, DMA, mechanical testing, water absorption and contact angle. The tannin-containing WPC presented lower interspaces between PP and lignocellulosic matrix, higher hydrophobicity and comparable mechanical performances. These results were due to the high compatibilization effect of the tannin, which allow getting similar results with 15% less lignocellulosic material. The lower crystallinity observed for PP in tannin-containing composites allowed a better contact between the wooden fractions. Although good physical compatibilization was obtained no evidences of chemical bonding were observed by FT-IR. The enhanced interphase contact and the higher hydrophobicity registered for the tannin-containing WPCs suggest that this natural, easily available extract can be a cheap and green additive to enhance the service-life for outdoor applications. POLYM. COMPOS., 00:000–000, 2017. © 2017 Society of Plastics Engineers

INTRODUCTION

Wood Plastic Composites (WPCs) are known since decades and they represent a very significant example of multi-functional materials, combining the advantages from natural, sustainable and low-cost lignocellulosic sources with the technological properties of the synthetic thermoplastic polymers [1, 2]. At present, the worldwide production of WPCs is around 2.5 million tons and 10% of it is dedicated to the European market. A further increase in the production of around 70% is expected for the coming years [3].

These composites are produced by extrusion, injection and molding at high temperature of the blend between classic polyolefin (e.g., PE, PP) and lignocellulosic fractions and therefore, they can be tailored for specific applications by modifying ratio and preparation conditions [4]. Within the last decades, a number of researches were successfully led to exploit different lignocellulosic sources

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such as sugarcane, ramie, jute, linen, pineapple, sisal, coconut, cotton fibers, heart-of-peach palm sheath, orange pruning fibers [5–7] for preparing bio-based plastic composites, although the majority of the commercial applications are still based on wood sawdust and fibers [8–12].

The wood-plastic composites can be used for several applications from general packaging to automotive components and in all the construction compartments from flooring, to cladding and decking of building [5, 13, 14]. The highest technological drawback for the preparation of the polypropylene/lignocellulosic composites is the limited chemical affinity between these two components. As a matter of fact, the polypropylene chains are non-polar polymers, while the lignocellulosic substrates are basically polar due to the high amount of hydroxyl groups of the carbohydrates and the lignin [15]. Looking towards improving the technical performance of wood-plastic composites, many research groups are searching for coupling agents/compatibilizer to improve the affinity between the aliphatic polymers and the lignocellulosic materials [16].

Traditional coupling agents are based on synthetic materials; polypropylene grafted with maleic anhydride is the most commonly applied example. It works due to the chemical anisotropic of this compound, allowing it to interact at same time with the polypropylene main chain and with the OH groups from the lignocellulosic materials [17–20]. In some studies, chemical modification of the lignocellulosic materials was also used to enhance the interaction with polypropylene [21, 22].

Besides those strategies, some natural macromolecules were already used to obtain better interaction between the two constituents. For example, technical lignin was successfully applied as stabilizer and compatibilizer in polypropylene composites [16, 23]. Chemical reactions between these natural macromolecules and polypropylene are not expected; however, since lignin contains both hydrophobic and hydrophilic functional groups; it acts as a bridge between the lignocellulosic and the polymeric matrix. However, lignin presents very high natural variability, which is further increased when different methods are used for its extraction from the plants because this process always involves cleavage and significant chemical modifications depending on the pulping process applied.

A less variable material of natural origin and with similar chemical properties is also abundantly available in the wood industry: The tannin extracts. In particular, the condensed tannin extracts are constituted by oligo-/polyphenols, which maintain similar composition, and therefore is possible to have a more reliable resource for compatibilizing the WPCs.

The condensed tannin from Acacia mimosa—Black wattle (*Acacia mearnsii* formerly *mollissima* de Wildt), in particular, is very abundant. According to the most recent statistics there are around 350,000 ha of black wattle forests worldwide and several local companies are extracting this resource with a production rate of over 200,000 tons of condensable tannins per year [24, 25], which are principally destined to the leather tannery processes and for the treatment of polluted water [26–28].

TABLE 1. Mass percent composition of the prepared composites.

Code	Fiber	Pine sawdust	Tannin	PP
P-PP	—	70	—	30
P-T-PP	—	55	15	30
F-PP	70	—	—	30
F-T-PP	55	—	15	30
Neat PP	—	—	—	100

P = pine sawdust; F = fibers; T = tannin; PP = polypropylene.

The polyphenols of the tannin extracts contain non-polar and polar functional groups: The aromatic part may present some affinity with the polypropylene, while the hydroxyl groups may interact with the lignocellulosic substrates through hydrogen bonds [29].

Considering that one of the major drawbacks of the WPC for construction purposes is their limited durability due to fungal and UV-radiation attack [30, 31], the tannin presents the intrinsic advantage of being the natural chemical protection system of the plant against these degradation agents [32–34]. Therefore, the addition of tannins could be the ideal solution for extending the service-life of these composite materials.

In this context, this study aims to incorporate tannin into flat-pressed WPCs produced with wood sawdust and fibers and to characterize these composites for their morphological, physical and chemical properties.

EXPERIMENTAL

Raw Materials and Preparation of the Composites

The tannic extract was obtained from the *Acacia mearnsii* bark through an industrial process based on treating the bark with water, heat and pressure for a certain time (SETA® private parameters). According to the industry data, the tannin extract is composed by 75% of the condensed tannins and 25% in hydrocolloid gums, sugars and small molecules such as the flavan-3-ol.

Pinus sp. Sawdust of 40–60 mesh were collected at the Embrapa Florestas sawmill, while the *Eucalyptus* sp. fibers of around 1 mm length were supplied by Duratex S/A. A matrix of polypropylene—PP H103 was supplied by Braskem (Brazil) with density of 0.905 g cm^{-3} and melt flow index of 40 g min^{-1} was used. The number-average molecular weight (Mn) of the PP is 49.44 Da and mean weight (Mw) is 235,597 Da.

Different mixtures of fibers or sawdust, polypropylene and tannin (Table 1) were homogenized using a thermo-kinetic mixer MH-100 equipment and then thermal molded by flat-pressing at 4 MPa, 175°C, for 15 min in a hydraulic press Marconi MA 098/A. A total of 90 g was used to each composite, with a nominal dimension of

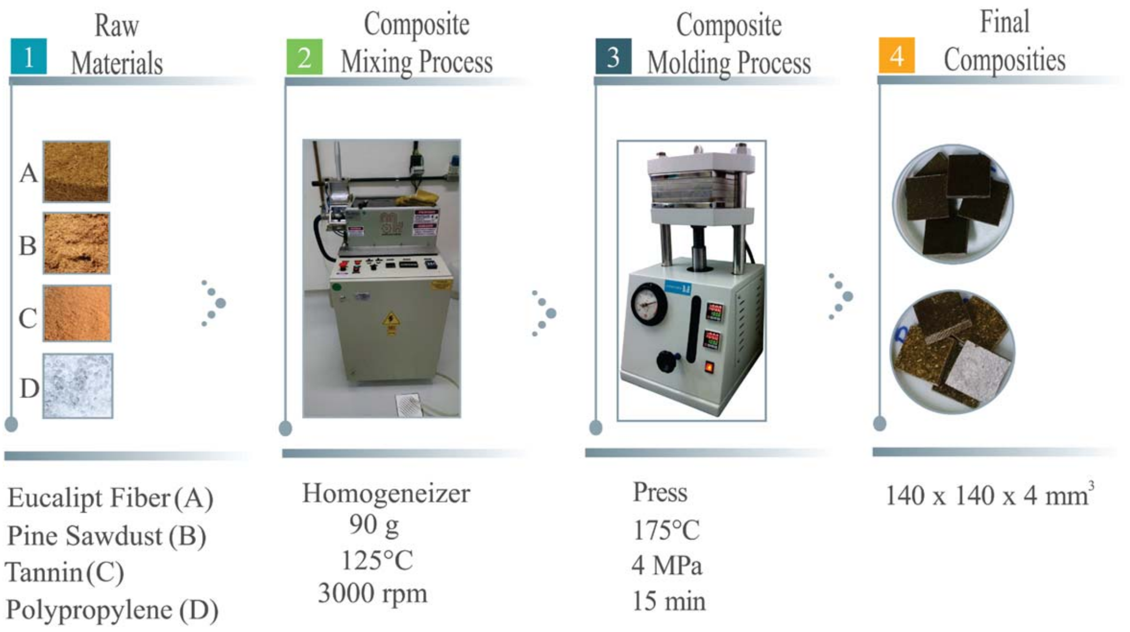

FIG. 1. Raw materials, process parameters and final composites obtained. [Color figure can be viewed at wileyonlinelibrary.com]

140 mm × 140 mm × 4 mm. In Fig. 1, the process of WPCs production is summarized.

Characterization of the Obtained Composites

Density Measurements. The density of the WPCs was measured following the procedures from ASTM D792—13 [35]. The density profiles of the WPC surface were performed using a Dense-lab X from EWS with samples of dimension 50 mm × 50 mm × 4 mm.

Scanning Electron Microscopy (SEM). The morphological features of the composites were elucidated using a desktop unit scanning electron microscope FEI Phenon. The composite samples were brittle fractured, and a thin layer of gold was deposited onto their surface.

Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA). TGA tests were performed in a Setaram equipment—SetSys Evolution model. Samples (~8 mg) were heated from 30°C to 600°C at a constant heating rate of 15°C min^{-1} under argon flow rate of 20 ml min^{-1}. The mass (TG) and mass loss rate (DTG) curves were determined.

DSC tests were carried out using a Shimadzu DSC-60 equipment. The samples were heated from 30°C to 190°C, at 10°C min^{-1}, and kept at isothermal condition for 5 min. After that, the samples were cooled to 30°C at the same rate. The data from DSC tests were used to obtain crystallinity index of the polypropylene.

Dynamic mechanical analysis (DMA) was performed with rectangular measuring system using a single cantilever. Rectangular samples were cut from the original composite to the size of 35 mm × 15 mm × 4 mm. The samples were measured using a TA Q800 instrument to obtain the curves of storage modulus (E') and loss factor (tan δ) at the fixed frequency of 1 Hz and temperature ranging from −130°C to 140°C, at a heating rate of 5°C min^{-1} under a nitrogen flow.

Mechanical Analysis. For the bending tests, five samples of 50 mm × 15 mm × 4 mm of each WPC series were tested using a Zwick/Roell Z 250 universal testing machine with a compression rate of 2 mm min^{-1} in a tailored three-point bending test, where the distance between the two bottom points was 35 mm and the compression was applied in the middle of the sample through a metal bar with 2 mm thickness. Bending strength and the modulus of elasticity (MOE) were then determined. The Brinell hardness of the WPCs surface was measured according to the EN 1,534 (2,000) test with an "Emco-test automatic" machine. The hardness of each WPC was measured in six different points.

Behavior Against Water. Equilibrium moisture content (EMC) of the samples was obtained after placing the WPCs in a climatic chamber at 20°C and 65% of relative humidity, until stabilization. Water absorption (%WA) and thickness swelling (%TS) were measured and calculated according to the procedures from ASTM D570—98 [36]. Eight replicates were used for these measurements.

Besides, the wettability of the composites was measured with a Drop Shape Analyzer—DSA25 equipment, via the sessile drop method. The water contact angle (WCA) was calculated 15 s after depositing a 5 µl droplet

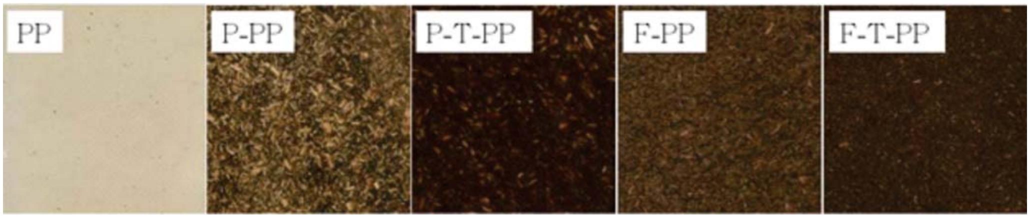

FIG. 2. Optical appearance of the wood-plastic composites. [Color figure can be viewed at wileyonlinelibrary.com]

of deionized water on the surface of acclimated composites samples (20°C and 65% of relative humidity). Spreading coefficient (SC) was calculated according Rothon [37] methodology. Five replicates were used for wettability determination.

Fourier-Transformed Infrared Spectroscopy. The wood-plastic composites were scanned with a Perkin-Elmer Frontier FT-MIR equipped of ATR Miracle diamond crystal. Each sample was scanned five times registering the spectrum with 32 scans with a resolution of 4 cm^{-1} in the spectral range between 4,000 and 600 cm^{-1}. The region between 1,800 and 600 cm^{-1} was evaluated after peak normalization and baseline correction.

The principal component analysis (PCA) was calculated by the software Unscrambler (Camo) considering the full range spectra. The cross validation method was used for the NIPALS algorithm.

RESULTS AND DISCUSSION

Material Distribution and Morphology

The wood-plastic composites, object of this research, were prepared according to the method already established for previous studies [8, 9] setting a target density of 1.15 g cm^{-3} for every formulation. The WPCs obtained had a flat and homogeneous distribution of the components as it can be observed in Figs. 2 and 3.

The optical appearance of the WPCs showed a homogeneous distribution of the lignocellulosic components in the PP matrix and in the composites containing tannin the color darkening also occurs homogeneously, implying that the distribution of the compatibilizer was correctly assessed. In Fig. 3, it can be observed that the distribution of the material along the thickness is also homogeneous and therefore the materials will have repeatable features. The effect of tannin in the vertical distribution shows slightly lower thicknesses and slightly higher densities in the core of the composite. The densities of all WPCs were very similar (1.129 ± 0.023 g cm^{-3}).

The SEM images of these WPCs showed that the presence of tannin allows the PP to establish a more intimate contact with the wood chips and fibers (Fig. 4). In the images with 10,000 magnifications, this effect is very well highlighted: the interspaces between the thermoplastic and the lignocellulosic matrixes appear significantly more fulfilled.

This observation is in line with the thickness decrease, because the molten polymer penetrates more deeply in the lignocellulosic substrate producing a local density increase.

Thermal Behavior

Thermogravimetric Analysis. The single components as well as the four composite materials have been analyzed for their thermal degradation from 30°C to 600°C (Fig. 5).

The analysis of the single components (Fig. 5a) allows to state that the tannin degrades much less than the PP and the two wooden fractions, especially when the temperature rises over 300°C. In particular, the mass loss at 600°C was only 50%. The polyphenolic structure of the Mimosa tannin is known to be particularly resistant against high temperatures [38–40].

Regardless the water desorption (<100°C), the main thermal degradation of the tannin took place in a mild way at around 250°C which ensures that no or negligeble thermal decomposition of the tannin will occur during the thermal molding of the composite (175°C).

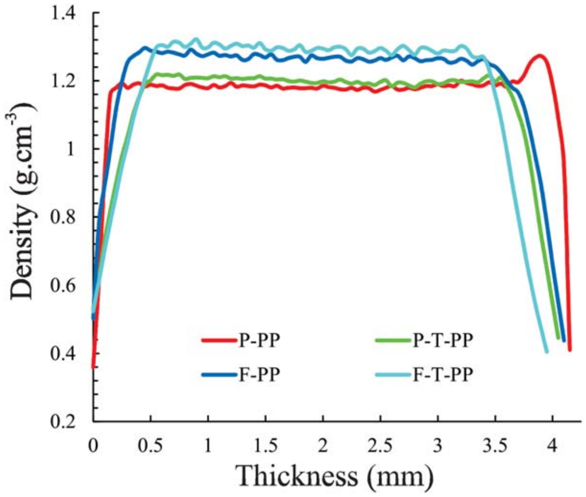

FIG. 3. Density profiles of the wood-plastic composites. [Color figure can be viewed at wileyonlinelibrary.com]

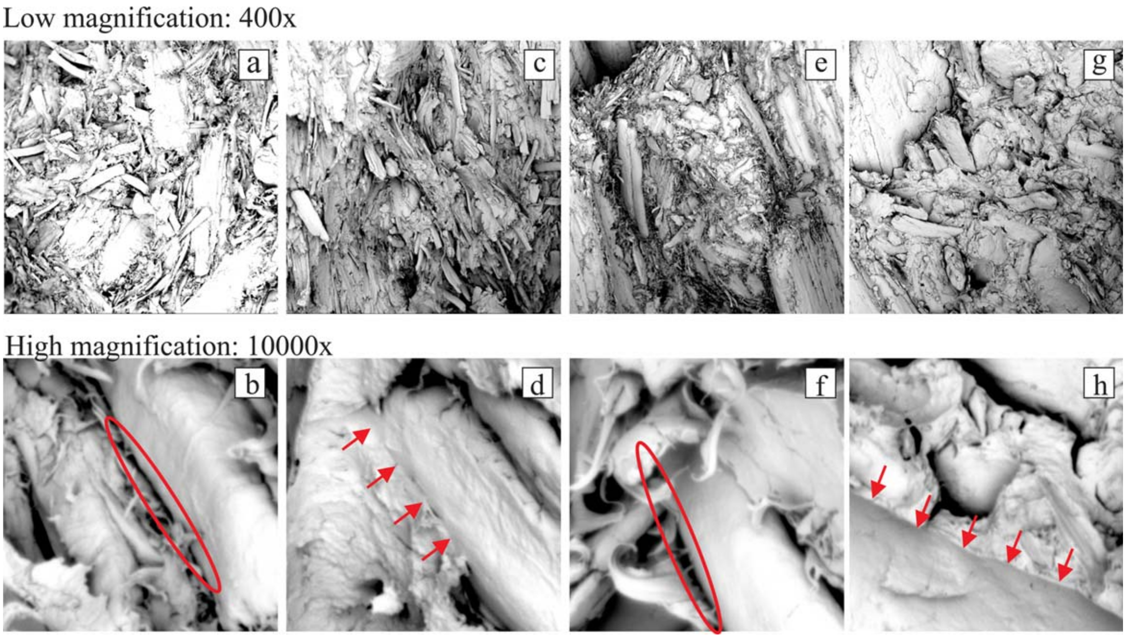

FIG. 4. SEM micrographs of composites' cross-section (a, b = P-PP; c, d = P-T-PP; e, f = F-PP; g, h = F-T-PP). [Color figure can be viewed at wileyonlinelibrary.com]

The wood fillers presented similar behavior; even if the pine sawdust presented a slightly higher mass loss between 400°C and 500°C (and a weak peak at ∼500°C), due to the higher lignin content in softwoods [41, 42]. However, the eucalypt fiber presented a shoulder at 270°C due to the thermal degradation of the hemicellulose. These two characteristic thermal behaviors are perfectly fitting with the observations of other researchers [9, 43, 44].

All composites presented similar thermal decomposition (Fig. 5c and d). The mass loss of the composites at 600°C is significantly lower than that of the components

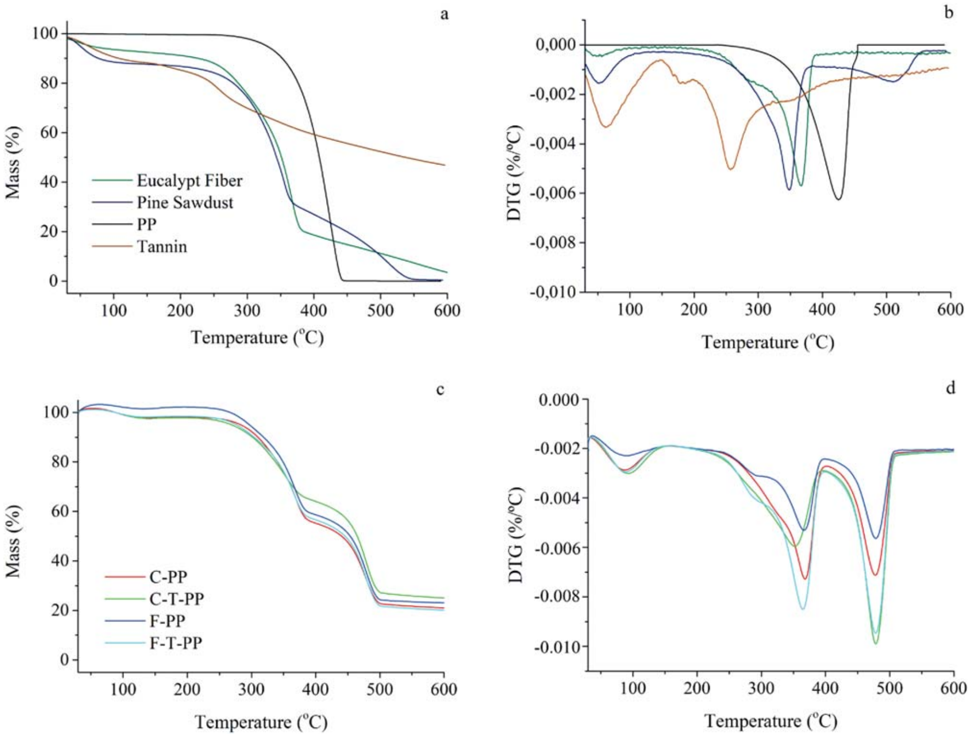

FIG. 5. Thermogravimetric curves and their first derivative of the raw materials (a, b) and the obtained composites (c, d). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. Summary of the DSC analysis of the composites.

DSC results	Neat PP	P-PP	P-T-PP	F-PP	F-T-PP
Melting					
Peak (°C)	166.1	163.5	164.1	163.5	165.2
T_{onset} (°)	157.0	156.7	156.7	158.9	159.6
T_{endset} (°)	170.5	169.9	168.9	168.6	170.3
Enthalpy (J/g)	−66.5	−15.3	−16.9	−13.6	−16.9
Enthalpy PP (J/g)[a]	—	−51	−56.3	−45.3	−56.3
Crystallization					
Peak (°C)	116.2	114.9	115.5	125.0	124.8
T_{onset} (°C)	120.8	119.8	119.4	128.6	128.7
T_{endset} (°C)	111.9	110.5	111.5	120.7	119.8
Enthalpy (J/g)	97.6	30.2	27.3	30.4	28.9
Enthalpy PP (J/g)[a]	—	100.6	91	101.3	96.3
Crystallinity (%)	51.4	53.0	47.8	53.3	50.7

[a] Enthalpy calculated using the proportional mass of polypropylene in the composite.

alone. The degradation profiles for all the WPCs are very similar and they can be easily explained by superposing the thermograms of the single components. The presence of tannin in the formulation renders the composite slightly early degrading when the temperature rises to around 200°C and 400°C, but resulting to a finally lower mass loss especially for WPCs with particles.

The crystallinity of the polypropylene measured with the DSC (Table 2) showed a decrease due to the presence of tannin in the composite from 53.0% to 47.8% for the P-T-PP and from 53.3% to 50.7% for the F-T-PP. This is a very interesting aspect suggesting that the tannin partially hinders the PP matrix to orient in a crystalline way, favoring an amorphous shape more suitable for combining with the surface of the wood fillers. This observation explains also why the PP in the tannin-containing WPCs begins to degrade slightly before.

Dynamic Mechanic Analysis. The dynamic mechanical measurements between 130°C and 140°C have shown the profiles reported in Fig. 6.

All the composites presented higher storage modulus than the PP. The graphic also show that the presence of tannin in the WPCs allow higher storage modulus at temperature <25°C and lower when the temperature rises over 75°C.

Since the storage modulus (E') gives results from the mechanical energy stored at the elastic-viscous phases and transition, it is possible to affirm that lignocellulosic materials (pine sawdust and eucalypt fibers) were efficient to improve the capacity of the polymer to convert mechanical stress into deformation. The tannin, in particular, increases this tendency before the glass transition temperature and decreased it after the viscous state is reached. This means that the tannin increases the energy storage of the composite at room temperature and then it contributes in weaken the material after the glass transition occurred. These observations are perfectly in line with the compatibilizing effect: (i) At low temperatures, the tannin-containing composites are more resistant because of the improved interconnection between the PP and the lignocellulosic substrate and (ii) at high temperature, the presence of lower amount of crystalline PP involves the composite to become viscous more quickly. These features can be very interesting when faster weakening of the polymer at high temperatures are requested, like in some events of fire where the affected part has to melt faster to avoid fire propagation.

Mechanical Properties. One of the key properties for the WPCs is their mechanical resistance and in Table 3 the measured values for the bending properties and the surface hardness are reported.

The Neat PP showed different mechanical performances than the ones obtained by the WPCs. The presence of lignocellulosic materials strongly increased the modulus of elasticity (of around 1/3rd) and the hardness (around double) of the material while the flexural strength decreased to 1/3rd. Similar results were already registered by other researchers [9, 45, 46]. Conversely, the results observed for the bending properties and for the surface hardness of the WPCs presented similar tendencies. In these cases, the tannin did not improve the mechanical

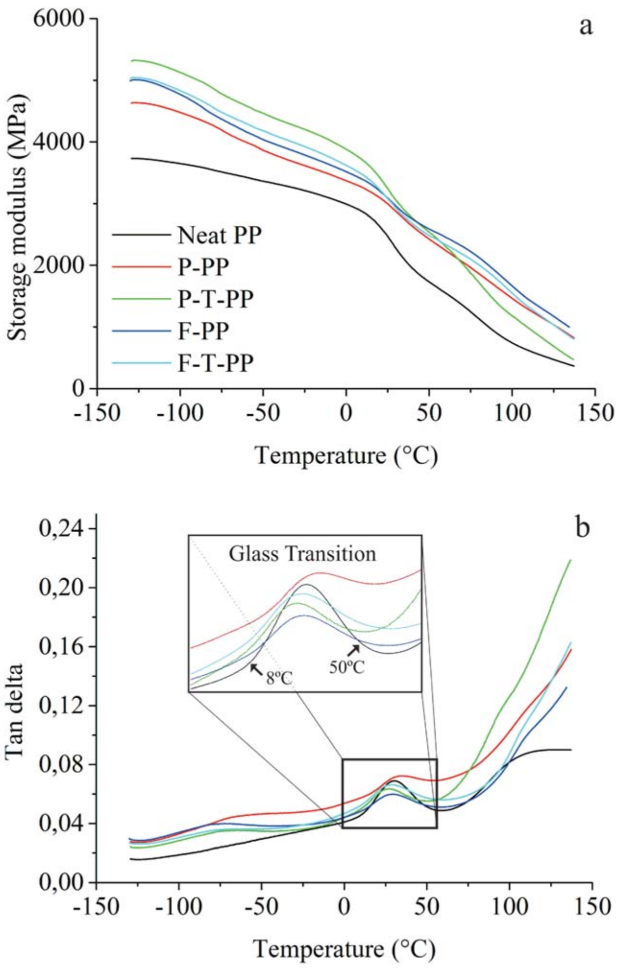

FIG. 6. Storage modulus (a) and tan δ (b) results of the composites and neat PP. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3. Mechanical properties of the obtained composites.

Sample	Modulus of elasticity (MPa)	Flexural strength (MPa)	Hardness Brinell (MPa)
Neat PP	1 529 ± 60 a	68.5 ± 4.1 b	48 ± 10*
P-PP	2 091 ± 169 bc	22.3 ± 3.7 a	110.0 ± 2.4 b
P-T-PP	2 033 ± 117 b	21.5 ± 1.2 a	104.3 ± 3.1 a
F-PP	2 295 ± 40 c	23.7 ± 0.5 a	106.5 ± 2.8 a
F-T-PP	2 046 ± 130 b	20.3 ± 1.1 a	104.1 ± 1.6 a
F-statistic	7.41*	181.30*	4.69*

Means ± standard deviation; significant at 5% of error probability ($p < 0.05$) according to LSD Fisher test; Average values in the same column followed by the same letter are not statistically different ($p < 0.05$, Fisher test); *Kaymakci et al. [46].

properties of the composites, but the performance lost observed were very contained. This result is very important because despite a significantly lower amount of sawdust and fiber, the bending properties as well as the Brinell hardness do not suffer significant decreases. We can state that the compatibilization with tannins allows to maintain very good performances even if 15% less of lignocellulosic fraction was used. Therefore, it is logical to figure out that specific tannin concentrations in WPCs may result in a compatibilized product with improved mechanical properties. This result is particularly satisfactory when compared with other natural compatibilizer that has shown 12% loss in flexural modulus with only 2% of lignin [16].

Behavior Against Water. The two components of the WPCs have opposite behavior against water. Polypropylene is absolutely hydrophobic while the lignocellulosic matrix is generally hydrophilic. Tannins are polyphenols and, hence, they present intermediate properties: The presence of hydroxyl groups ensures a certain hydrophilicity but the presence of aromatic in the structure smoothen this trend. In Table 4, several parameters explaining the relation of the WPCs with water such as the equilibrium moisture content (EMC), the water absorption (WA) and thickness swelling (TS) after 2 and 24 h as well as the WCA and the SC are reported.

As expected, the water affinity for the WPCs was higher than that of neat polypropylene, but very limited hydrophilicity was observed for all the composites. The WPC having higher water affinity was the P-PP because of the presence of wood particles on the surface exposing a higher surface directly to the wet environment and therefore being subjected of higher EMC, WA and TS. It is interesting to remark that the performances of the P-T-PP were more hydrophobic. This can be explained by two facts: (i) there is less lignocellulosic material to be hydrophobized and (ii) the decrease in crystallinity of the PP due to tannin. Indeed, an amorphous material wet better a surface than a crystalline one, or in other words, when the PP contains tannin it can more homogeneously distribute around the lignocellulosic matrix and therefore the surface of the particles offered directly to the wet environment was lower. This observation allows to understand all the improvements and confirms the compatibilization effect of tannins. For the composites with the fibers, this effect did not occur because the fibers are easier to be wet and therefore, the lignocellulosic surface directly exposed to the wet environment is lower.

The increase in amorphous PP due to tannin, particularly highlighted for the WPCs with particles, allowed better wettability of the lignocellulosic substrate with consequent hydrophobization of the WPC surface. This enhancement may result very interesting for all the outdoor applications of these materials, because a lower water contact determines also a better resistance against biologic degradation (e.g., insects, mold, fungi).

Infrared Spectroscopy Study. The infrared spectra registered for the pine sawdust and fiber-based wood polymer composites showed that their vibrational fingerprint is a perfect linear combination of the infrared signal from the constituent components (wood and PP) and therefore,

TABLE 4. Density and water/composites relationship results of obtained composites.

	Sample					
Response	Neat PP	P-PP	P-T-PP	F-PP	F-T-PP	F-statistic
EMC (%)	0.2 ± 0.05[#]	10.5 ± 1.5 b	5.7 ± 0.7 a	5.2 ± 0.6 a	5.8 ± 0.2 a	39.2*
WA-2 h (%)	—	0.8 ± 0.2 b	0.5 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.1 a	7.4*
WA-24 h (%)	0.03**	3.1 ± 0.5 c	2.5 ± 0.1 b	2.1 ± 0.1 a	2.5 ± 0.1 b	11.4*
TS-2 h (%)	—	1.4 ± 1.2 b	1.0 ± 0.5 ab	0.3 ± 0.1 a	0.3 ± 0.1 a	3.5*
TS-24 h (%)	0.09[$]	3.7 ± 2.0 b	2.7 ± 0.9 ab	1.7 ± 0.7 a	1.7 ± 0.4 a	3.4*
WCA (°)	85.3 ± 4.0 c	77.2 ± 2.3 a	75.8 ± 3.9 a	78.8 ± 3.5 a	77.0 ± 3.2 a	12.0*
SC	−67.7 ± 5.0 a	−57.5 ± 2.8 b	−55.7 ± 4.8 b	−63.6 ± 7.1 a	−54.2 ± 4.6 b	12.5*

Means ± standard deviation. Values having the same letter are not significantly different. *Fisher LSD test ($p < 0.05$). [#]Shakeri and Ghasemian [47]; **Deng et al. [48]; [$]calculated by the Shakeri and Ghasemian [47] equation.

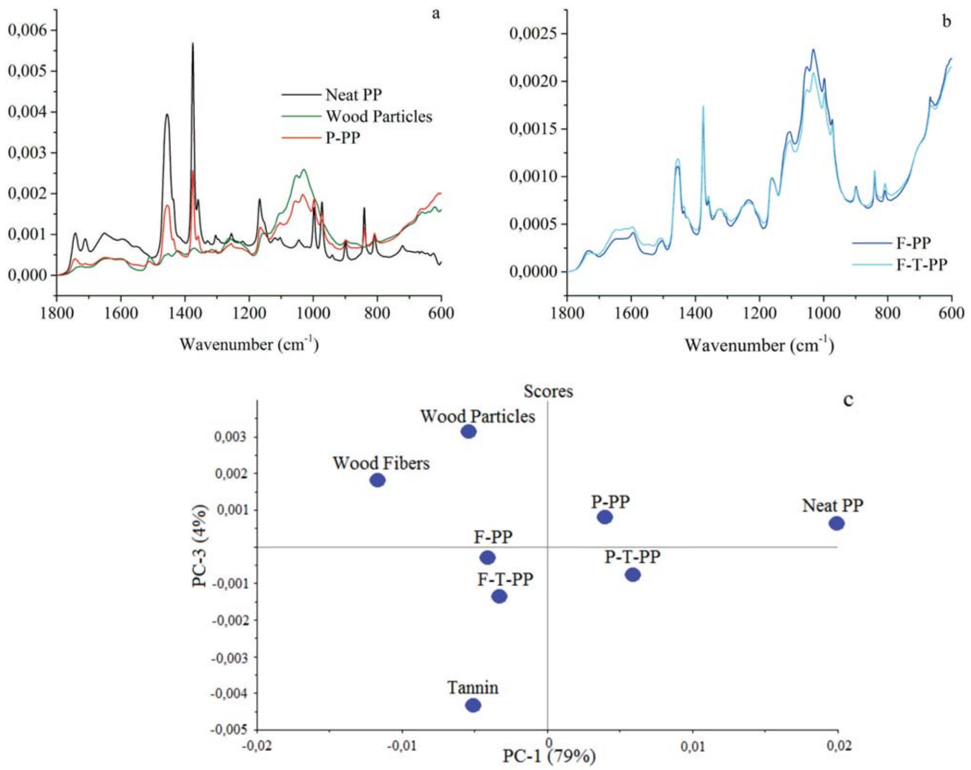

FIG. 7. Infrared spectra of pine sawdust and pine sawdust-based wood polymer composites (a), fiber and tannin-compaptibilizer fiber composites (b) and PCA of the ATR-FT-MIR data(c). [Color figure can be viewed at wileyonlinelibrary.com]

no new chemical bonding can be foreseen (Fig. 7a and b).

The only observable phenomena was the presence of tannin due to the increase in the signals in the area between 1,700 and 1,500 cm^{-1} and weakened the absorbances in the region between 1,100 and 900 cm^{-1}. For both lignocellulosic sources, new absorptions are not occurring, which suggests the simple blending between the three components.

To confirm this observation, the PCA presented very interesting data distribution in the graphic were PC1 (79%) and PC3 (4%) are reported (Fig. 7c). It was easy to observe that PP and the lignocellulosic materials stay in opposite regions of the PC1 while the wood and the tannin stay in opposite region of the PC3. All wood-plastic composites stay in correctly distributed intermediate positions. Therefore, the PCA score plot confirms that composites are simple blend of its constituents and chemical bonding between PP and the lignocellulosic fraction did not occur.

Even though no chemical crosslinking can be observed, it can be stated that the mimosa tannin is a good compatibilizer for WPCs because it decreases the possibility of PP to arrange in a crystalline way and therefore it will more intimately interconnect with the lignocellulosic matrix. The tannin-containing WPCs result: (i) structurally more compacted; (ii) thermally more resistant before 25°C and weaker after 75°C; (iii) mechanically comparable and (iv) more hydrophobic than the respective tannin-free homologues composites.

The properties improvements obtained by adding tannin allow to consider these materials for plenty of purposes. The principal advantage expected is the enhancement of the service-life when the WPCs will be used for outdoor applications. The presence of tannin allows a better distribution of the PP over the matrix, implying lower direct exposure of wood to the external environment and hence lower accessibility for water and oxygen in the interspaces. This will decrease the degradation process by biologic attack (white, brown & soft rots and also mold) and it will decrease the degradation due to the UV-light exposure. Combined to this lower accessibility of the wooden matrix, tannins have natural preservation properties against fungi and they act as radical scavenger for the protection against UV-light exposure [33, 49, 50]. Biological tests of this material to confirm the increase in durability are already started.

The thermal behavior suggests that the presence of tannin allow the material to be used in a wide range of temperatures and only when the 75°C is reached the material become more easily melted. These temperatures can be obtained quickly in case of fire and the tannin-containing WPC will melt faster which could be ideal for the containing fire in a limited area. Furthermore, the presence of tannin will restrain the burning because of its particular self-extinguishing properties against fire [51, 52]. A final further advantage of the tannin-containing WPCs is that they are darker, resulting in a more attractive color for different products like door and windows frames as well as for decking, cladding and flooring of buildings.

CONCLUSIONS

WPCs containing tannin have been successfully produced. Even if no chemical interaction occurs between the tannin, the PP and the lignocellulosic fiber, the flavonoids acts as good physical compatibilizer because they allow a better distribution of the polypropylene over the lignocellulosic filler. For this reason, the new materials resulted more hydrophobic than the classic WPC and easier to melt at temperature higher than 75°C because of the lower crystallinity degree of the PP. The mechanical performances of the tannin-containing WPCs were very good in terms of storage modulus and satisfactory in bending properties comparing them to the WPC without tannin, which contained 15% more lignocellulosic material, the major responsible for these properties. These findings promote the tannin as an interesting, cheap and green compatibilizer, especially when lower water affinity is requested and particularly every time the product would be suited in outdoor environment. Theoretically, the tannin would contribute in protecting the WPCs twice: (i) decreasing the direct contact between the wood and the outdoor environments and (ii) by working as active protecting agent against fungi and UV-lights.

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5. MANUSCRIPT 4

NANOCELLULOSE-TANNIN FILMS: FROM TREES TO SUSTAINABLE ACTIVE PACKAGING

Filmes de nanocellulose-tanino: de árvores para embalagens ativas sustentáveis

RESUMO

O que pode ser mais sustentável do que proteger os nossos produtos com derivados de árvores? A celulose e o tanino podem oferecer proteção física e química, respectivamente, para obter uma embalagem “ativa” do futuro. Nesse trabalho, nós relevamos os segredos da incorporação do tanino em um filme de nanocellulose (CNF) e as propriedades desse novo material foram investigadas. Os filmes de nanocellulose com tanino (CNF-T) mostraram densidades muito elevadas e um aumento na hidrofobicidade superficial, sugerindo acréscimos nas propriedades de barreira. Esses filmes mostraram também excelente capacidade antioxidante e resistência contra solventes químicos. Essas importantes propriedades são devidas a uma interação química íntima entre os dois recursos biológicos, sendo alterada apenas quando o novo material entra em contato com a água. Dessa forma, os filmes CNF-T apresentaram todas as características importantes para serem considerados como um novo material para embalagens “green” e ecológicas para alimentos e produtos farmacêuticos.

Palavras-chave: Polifenóis, NFC, *Acacia Mearnsii,* molhabilidade, validade, preservativo.

Nanocellulose-tannin films: from trees to sustainable active packaging

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ABSTRACT

What can be more sustainable than protecting our goods with tree derivatives? Cellulose provides the physical and tannin the chemical protection to obtain the “active packaging” of the future. In this work, the secrets of successfully embedding the tannin in a cellulose nano-fibrillated (CNF) film are revealed and the properties of these innovative materials are investigated. The tannin-added CNF (CNF-T) films show very high densities and strongly improved surface hydrophobicity which suggest high barrier properties. These films show also an outstanding antioxidant activity and a strong resistance against solvents. These properties are due to an intimate chemical interaction between the two bio-resources which result altered only when the material get in contact with water. The CNF-T films present all the most important characteristics to be considered as a valid green and eco-friendly packaging material for foods and pharmaceuticals.

KEYWORDS: polyphenols; NFC; *Acacia Mearnsii;* wettability, shelf-life, preservative.

1. Introduction

Almost all the items on the market are packaged. The packaging plays the fundamental role of protecting the goods from physical damages, contamination and deterioration (de Léis et al., 2017; Ingrao et al., 2017; Wang and Wang, 2017). Synthetic plastic such as low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE) are the favored materials for packaging because they are light, inert and easily available. That´s one of the reasons why the production of plastics has increased exponentially over the last 65 years (Bernstad Saraiva et al., 2016). In Europe, for example, the production of oil-derived plastics reaches 49 millions of Tons/year of which almost 40% is used for packaging purposes (Plastic, 2017).

Although the traditional packages fill the needs of containing and protecting, for some applications a new property is gaining importance: the interaction with the good. A new kind of packaging namely the “active” packaging was proposed. These materials combine to the traditional packaging features also a specific chemical protection against oxidation, moisture and UV rays, for instance. This active extra protection increases the shelf-life or the expiration date of the goods, and it can absorb unwanted substances such as heavy-metals or exhausted oils, or even release protective chemicals like preservatives or antioxidants (EU, 2009).

In the last years, the research in the field of active/sustainable packaging is strongly increased (Beitzen-Heineke et al., 2017; Chen et al., 2016; Mekonnen et al., 2016; Moustafa et al., 2017; Razza et al., 2015): Active packaging with synthetic films of polycarbonate and polyethylene added of phenolic substance (Krepker et al., 2017) and inorganic compounds of copper and zinc (Al-Naamani et al., 2016; De Vietro et al., 2017) have been successfully presented as antimicrobial packaging. Very recently, greener films based on carboxymethylcellulose - chitosan - oleic acid added of zinc oxide (ZnO) were considered suitable for prolonging the shelf-life of sliced wheat bread (Noshirvani et al., 2017).

Future trends indicate that a new frontier of research in this field is the synthesis of active packaging using green and sustainable resources. Nature made the work for us: The trees provides already the substances we need to produce the active package of the future: cellulose and tannin.

With its estimated 10^{11} Tons/year, cellulose is the most abundant bio-macromolecule on Earth and has been used for all sorts of purposes for thousands of years (Douglass et al., 2016). Cellulose nano- and microfibrils are strong, lightweight, chemically versatile, non-toxic and biodegradable (Dufresne, 2013; Lundahl et al., 2017). Its peerless properties are fundamental for a broad range of applications that go from common paper, to chemically modified cellulose (Belgacem and Gandini, 2008), from polymer matrix for high-tech electronic supports to nano-fibrous material for optical devices (Cai et al., 2016; Duan et al., 2016). Cellulose can also be nanofibrillated (CNF) through mechanical friction to produce chains with diameter of 5-60 nm and some µm of length which improve the flexibility of the material (Hubbe et al., 2017). The CNF can be arranged in films with excellent modulus of elasticity of 10-150 GPa (Iwamoto et al., 2009; Lee et al., 2012) and very high barrier properties (Hubbe et al., 2017; Hult et al., 2010; Lavoine et al., 2012) and therefore they were successfully proposed as bio-based packaging material (Amini et al., 2016; Lavoine et al., 2014; Lavoine et al., 2015; Lavoine et al., 2016).

With about 200 thousand tons/year corresponding to around 90% of the worldwide tannin production, the condensed tannins are the most abundantly extracted natural substances in the Earth (Pizzi, 2008). The bark of *Acacia mearnsii*, commonly known as black wattle or mimosa, is the most important source of commercially available condensed tannins (Duval and Avérous, 2016). These polyhydroxyphenols have a strongly antioxidant and antimicrobial properties (Missio et al., 2017b; Wei et al., 2015), and they have already been successfully applied for the biological protection of wood (Thévenon et al., 2010; Tondi et al., 2012) and as bio-active compatibilizer in wood plastic composites (Missio et al., 2017a).

Therefore, the goal of our research is to prepare a tannin-activated CNF films using only natural, renewable and sustainable resources and, successively to characterize its chemical, mechanical and physical properties. We have tried to prepare the film using innovative methodologies of net-making: (i) addition of tannin in the nanocellulose gel and (ii) co-grinding of the components. These different hydrogels were analyzed by transmission electron microscopy (TEM) while the derived films were extensively investigated through Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), apparent contact angle, thermo-gravimetric analysis (TGA), antioxidant activity, tensile strength and chemical resistance.

2. Material and methods

2.1.Raw materials

The active films were prepared using nanofibrillated as structural net material and wood tannin as the bioactive molecules source. The nanofibrillated cellulose was prepared according to our previous work (Kumode et al., 2017). Briefly: bleached Kraft pulp (from Suzano Celulose e Papel®) was turned into fluffy-like cellulose by using a high-shear mixer MH-100. Then, a 2 wt.% cellulose aqueous suspension was passed through an ultra-fine friction grinder (Masuko Sangyo Super Masscollider) at 1500 rpm until a homogeneous hydrogel containing nanocellulose fibers with diameter from 10 to 40 nm was obtained. The tannin we used here was obtained from *Acacia mearnsii* bark (from SETA® industrial process), and it is composed by 75% of the condensable tannins and 25% hydrocolloid gums, sugars and other small molecules such as the flavan-3-ol (Arbenz and Averous, 2015).

2.2.Preparation of active films

The process of production of nanocellulose films with and without tannin is summarized in Fig. 1. We tried two strategies to incorporate tannin into the nanocellulosic films. The first one was (i) 100g of tannin powder were manually mixed with approximately 1000g of nanocellulose gel and stirred for 5 min. The second and successful one was (ii) 100g of tannin was mixed with initial solution of fluffy cellulose directly in the friction grinder so that nanofibrillation process occurred in presence of tannin.

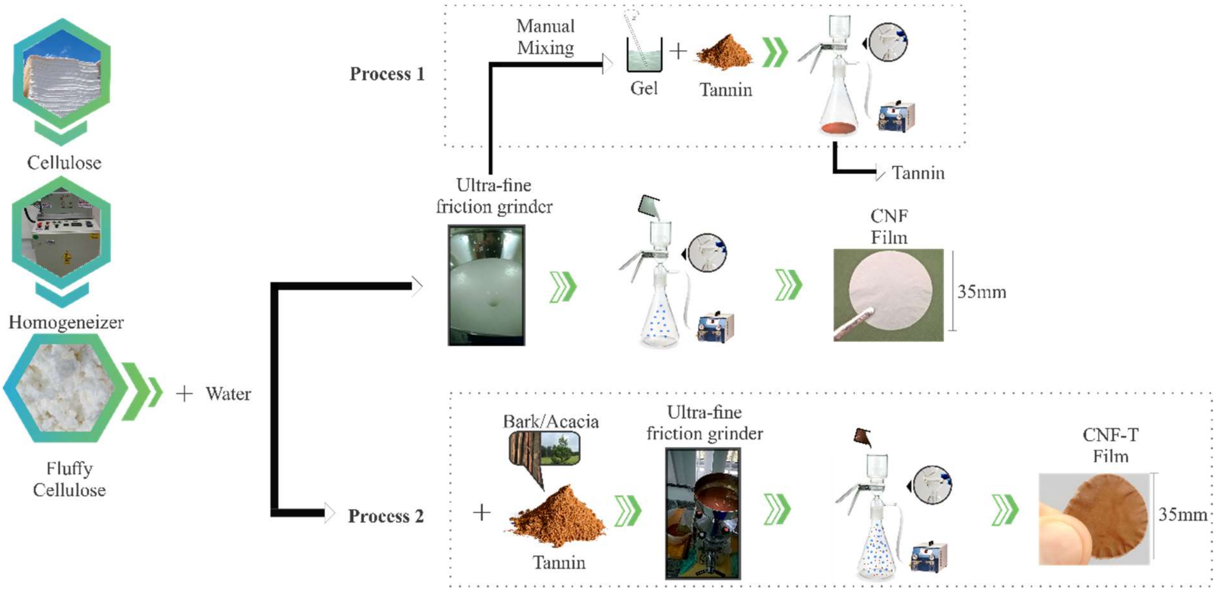

Fig. 1. Scheme of preparation of the nanocellulose films.

A filtration system (Whatman®) with a nylon membrane (diameter = 47 mm; porosity = 0.22 µm) connected with a vacuum-pump was used to produce the films. 1.4 g of gel were mixed with 50 mL of distilled water and deposited on the nylon membrane in order to obtain films with a target grammage of 30 g/m^2 and diameter of 35 mm. The films were air-dried for 4 hours, removed from the membrane and finally stored in climate chamber at 20°C and 65% relative humidity.

2.3.Morphology studies

The morphologic features of the nanocellulose hydrogels with and without tannin were observed using a transmission electron microscope (TEM) JEOL, model JEM-1200 EXII. For this, an aqueous suspension at 0.1% w/v of the hydrogels were dispersed in an ultrasonic bath for 30 min, and then dropped on a copper TEM sample grid. In addition, the films surface was observed by scanning electron microscopy (SEM) using accelerating voltage of 5 kV (Tescan, VEGA-3SBU, Czech Republic). All samples were coat-sputtered with Au to ensure the conductivity of the sample.

2.4.Density measurement

The density of the films was calculated measuring the weight and volume of 10 films per formulation. Weight was obtained thought analytic balance. The diameter of the films were obtained using a digital caliper and the thickness were measured using the procedure T 411 om-97 (TAPPI, 1997).

2.5.FT-IR analysis

The films were scanned with a Perkin-Elmer Frontier ATR-FT-MIR equipped of attenuated total reflectance (ATR) Miracle diamond crystal. Each sample was scanned 3 times registering the spectra with 16 scans with a resolution of 4 cm^{-1} in the spectral range between 4000 and 600 cm^{-1}. The region between 1800 and 600 cm^{-1} was evaluated after baseline correction, area normalization and averaging the measurements.

2.6.Thermogravimetric analysis (TGA)

TGA tests were performed in a Setaram equipment - SetSys Evolution model. The samples (~8 mg) were heated from 30 to 600°C at a constant heating rate of 15°C.min^{-1} under argon flow rate of 20 ml.min^{-1}. The mass loss (TG) and the first derivative (dw/dt) curves were determined.

2.7.Wettability measurements

The wettability of the composites was measured with a Drop Shape Analyzer – DSA25 equipment, using the sessile drop method. The water contact angle was calculated from 5 to 60 seconds after depositing a 20 μL droplet of deionized water on the surface of the samples. The measurements were replicated 10 times, and the results were reported as average with their relative standard deviation.

2.8.Antioxidant capacity

The determination of the antioxidant activity of the films was carried out according to the method described by Kaya et al. (2017). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich, USA), 2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH, Sigma-Aldrich, USA) and sodium fluorescein (Sigma-Aldrich, USA) were used. Monobasic and dibasic potassium phosphate (Vetec, Rio de Janeiro, Brazil) were used to prepare buffer (pH 7.4), and working solutions of AAPH, Trolox and sodium fluorescein. Ultrapure water was obtained using a MilliQ system (18.2 MΩ·cm, Millipore Corp., Bedford, MA). The fluorescence monitoring for ORAC assay was carried out using microplate reader (Sense, HIDEX, Turku, Finland).

The antioxidant capacity was evaluated in triplicate by Oxygen Radical Absorbance Capacity (ORAC) method (Ou et al., 2001). For this analysis, 100 mg of the film were submerged in 10ml of distilled water and the antioxidant capacity was measured in this water withdrawing 1mL after 1, 4, 6, 8, 12, 24, 36 and 48 hours. The samples and Trolox were diluted in potassium phosphate buffer 75mmol L^{-1} at concentration of 50 mg L^{-1} of sample and the Trolox at different concentrations (4 to 98µM). In a black microplate (96 wells) was added 25 µL of each sample and Trolox solutions and 150 µl of fluorescein (81 nmol L^{-1}), incubated at 37°C for 10min. Right after, was added 25 µL of AAPH solution (152 mmol L^{-1}) as a peroxyl radical generator. The fluorescence was monitored (excitation and emission wavelengths were 485±10 nm and 535±20 nm, respectively) in a microplate reader with 120 measuring cycles during 120 min at 37°C. The ORAC values expressed as equivalent of µmol of Trolox per g of sample were based on the Area Under the Curve (AUC) of fluorescence decay over time, calculated as described in equation:

$$\mathrm{AUC}=1+{}^{f1}/_{f0}+{}^{f2}/_{f0}+{}^{f3}/_{f0}+\dots{}^{fn}/_{f0}$$

in which $f0$ is initial fluorescence (t = 0) and fn is the fluorescence obtained in a read cycle.

2.9.Tensile strength & chemical resistance

The tensile strength of films stripes 30x10 mm^2 was measured using a Zwick/Roell Z 250 universal testing machine equipped with paper tensile test apparatus at the rate of 2 mm/min according to the DIN EN31 (DIN, 1993). Five measurements per formulation were tested and reported as average with their relative standard deviation.

The chemical resistance of the films was observed by dipping the films in different chemicals for 7 days according to the ASTM D543-14 (ASTM, 2014). Distilled water, ethanol, ethyl acetate, hexane, vegetal corn oil, sodium hydroxide (10%) and sulfuric acid (30%) were used to tests the chemical resistance. Sample weight and tensile strength measurements were used to quantify the chemical resistance. The tests were performed in triplicates.

3. Result and Discussion

3.1.Preparation of the nanocellulose-tannin films

Embedding tannin in the nanocellulosic net was performed using two different procedures.

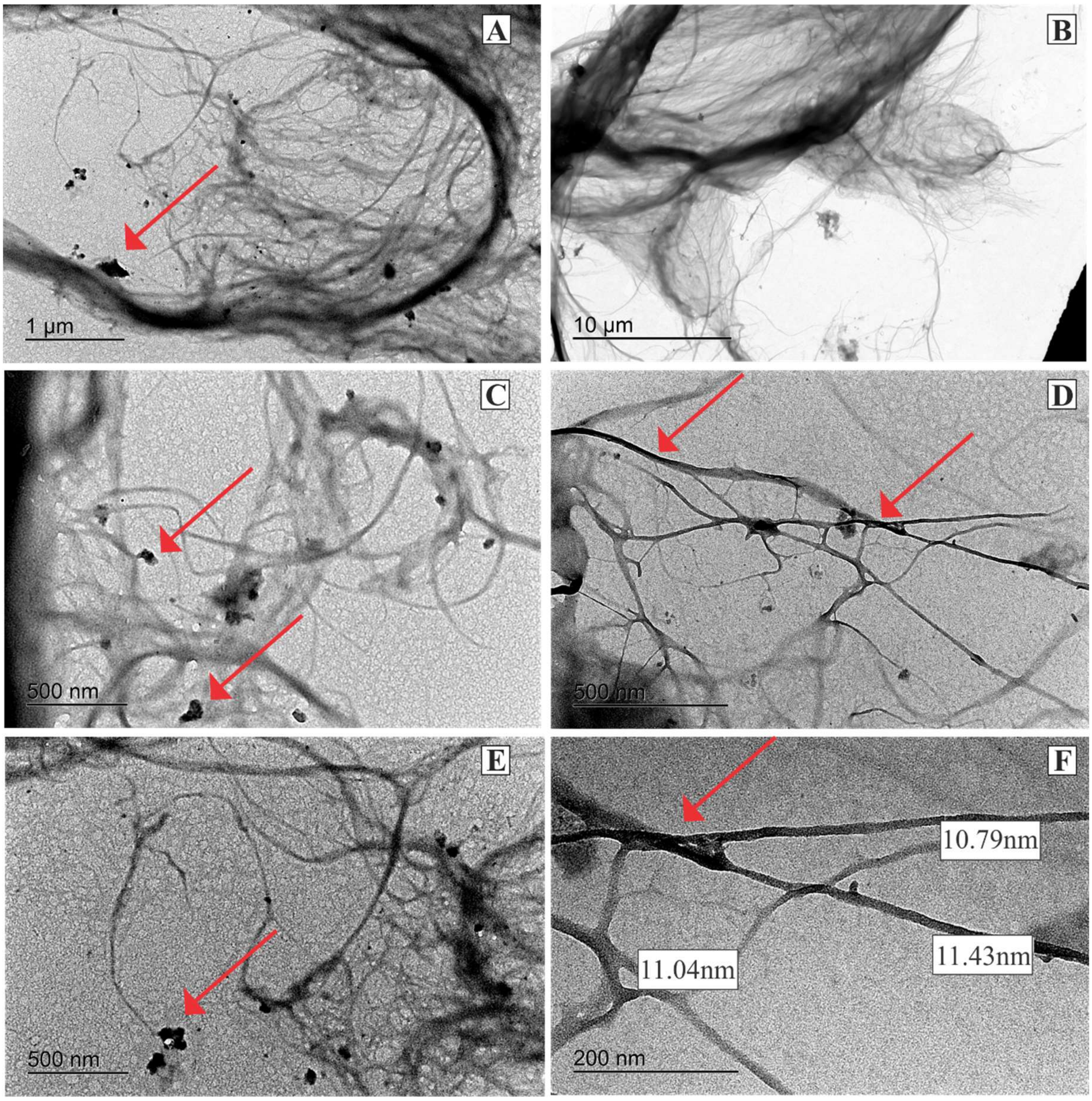

Fig. 2. TEM observations of the cellulose gel added with tannin extracts at different magnification. A, C, E - Tannin added after cellulose gel production; B, D, F - Tannin added before milling.

When the tannin was added after the formation of a cellulose gel and mixed by stirring (Fig. 1, process 1), the polyphenolic material resulted dispersed among the nanofibrillated cellulose, but still in form of colloids which appear completely distinguished to the cellulosic fiber (Fig. 2 A, C, E). Conversely, when the cellulosic hydrogel was ultra-fine grounded in the presence of tannin (Fig. 1, process 2), the resulted tannin-containing cellulose hydrogel showed an intimate connection between the tannin and the cellulosic fibers (Fig. 2 B, D, F). These two procedures had dramatically different consequences after filtering: The films prepared in the first way lose all the tannin during the filtration and resulted of white color, while the film produced according to the second method kept the tannin and appeared brown (Fig. 1). Hence, the joint ultra-fine grinding process of tannin and cellulose plays a fundamental role for a successful intimate interconnection between the two bioresources.

The latter brown film called nanocellulose-tannin (CNF-T) is the subject of this work and it is compared with the nanocellulose (CNF) film produced without tannin.

3.2. Chemical composition

One of the most important information to be considered for the CNF-T films is their tannin content. In Table 1 the information related to the production of the films are reported.

Table 1. Film composition.

Film	Initial amount of solid (mg)	Final weight of film (mg)	% Tannin in the film
CNF	$28.8^{\pm1.0}$	$24.5^{\pm1.2}$	0.0
CNF-T	$172.8^{\pm5.0}$ (28.8 CNF + 144.0 T)	$30.3^{\pm1.0}$	$19.6^{\pm4.57}$

The films produced with tannin result 23.7% heavier than the tannin-free films which means that the 19.6% of tannin are added to the nanocellulose which result equivalent in terms of weight for both films.

The FT-IR analysis of CNF, CNF-T and tannin powder is reported in Fig. 3.

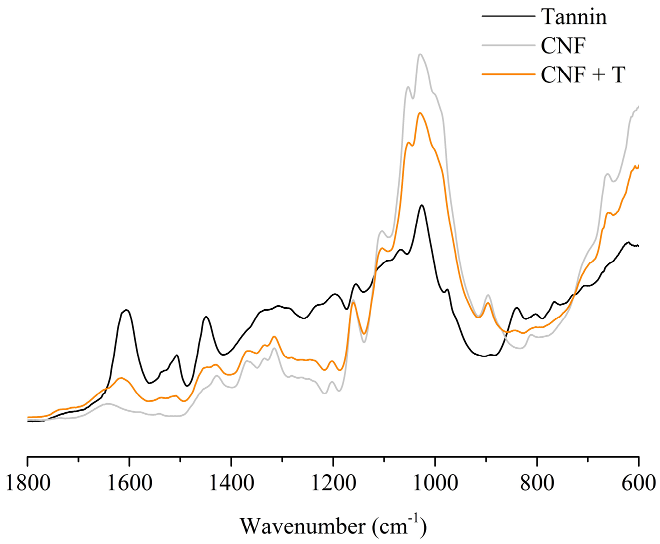

Fig. 3. FT-IR spectra of films of nanocellulose with (orange) and without tannin (grey).

The spectrum of the nanocellulose film (CNF) presents the typical absorption in the regions 1440-1400 and 1390-1290 cm^{-1} which can be attributed to C-H bending and wagging and a broad complex band 1200-900 cm^{-1} which is mainly due to the C-O and C-O-C stretching of the carbohydrate (Chung et al., 2004; Schwanninger et al., 2004).

The spectrum of the tannin-containing film (CNF-T) shows a very similar trend, but the peaks at 1605, 1510 and 1450 cm^{-1} assigned to aromatic/phenolic vibrations of the mimosa tannin, result enhanced (Tondi and Petutschnigg, 2015). This interpretation suggests that tannin is embedded in the cellulose net but limited or no covalent bonding are produced between the tannin and the nanocellulose.

3.3.Barrier properties

3.3.1. Surface morphology and density

The films produced present very similar morphology (Fig. 4). The SEM of the surfaces show a very compact material at microscopic level for both magnitude degrees observed. The films containing tannins presented some spots that appear well embedded in the nanocellulosic tissue. Other research groups have observed that soy-protein films embedded tannin presented enhanced of compactness due to the establishing of hydrogen bonds and hydrophobic interactions between the two bioresources (Wang and Wang, 2017). Even if the cellulose is a completely different substrate, it can be assumed that the film preparation process promotes intimate chemical interaction between materials through optimization of their secondary forces.

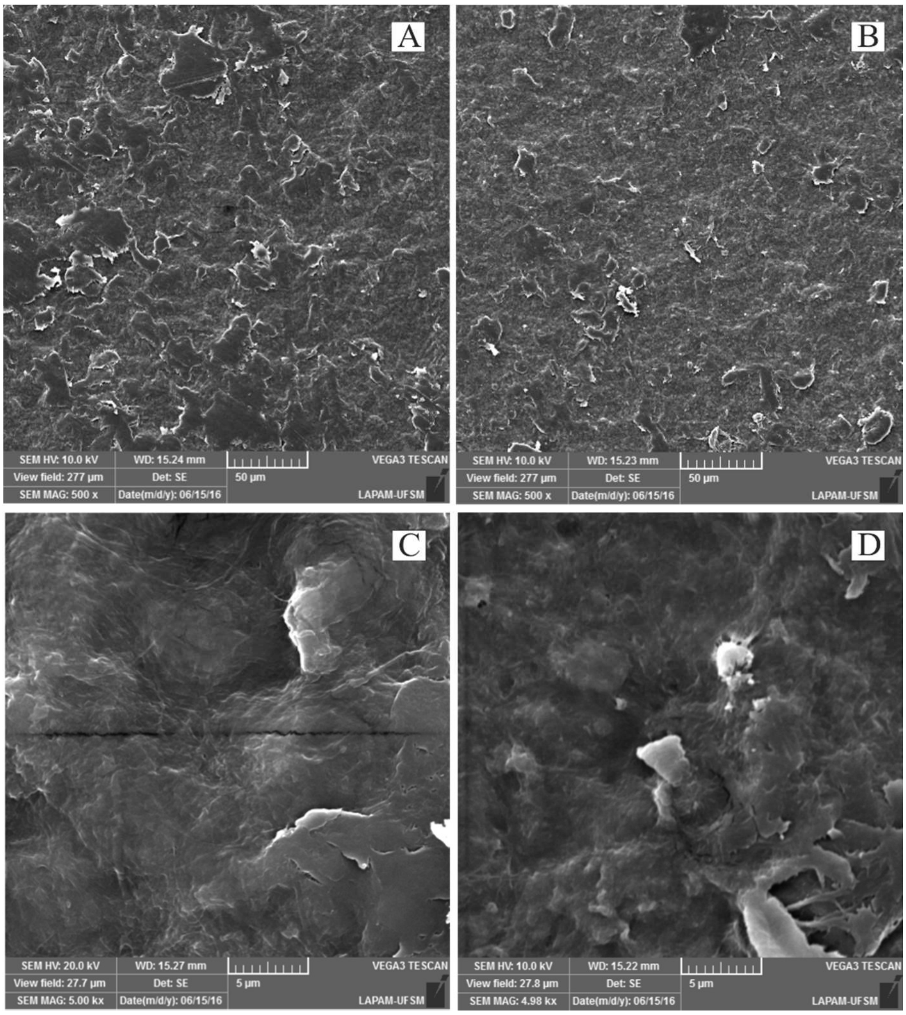

Fig. 4. SEM observation of films of nanocellulose without (A, C) and with tannin (B, D).

The high densities were also confirmed with micrometric measurements which showed slightly higher values of 1.50±0.15 g/cm³ for the nanocellulose films without tannins (1.44±0.21 g/cm³). These results are comparable with that of the cellulose nanofibrils (1.5-1.6

g cm^3) (Dufresne, 2013) which means that the produced films have low porosity and high compactness and consequent high resistance against air permeation (Aulin et al., 2010; Belbekhouche et al., 2011; Kisonen et al., 2015).

3.3.2. Apparent contact angle

High barrier properties are characterized by high densities, but for nanocellulose films, a limitation is represented by its good water affinity (Hubbe et al., 2017). The CNF-T films have shown improved properties against water penetration through apparent contact angle measurements (Fig. 5).

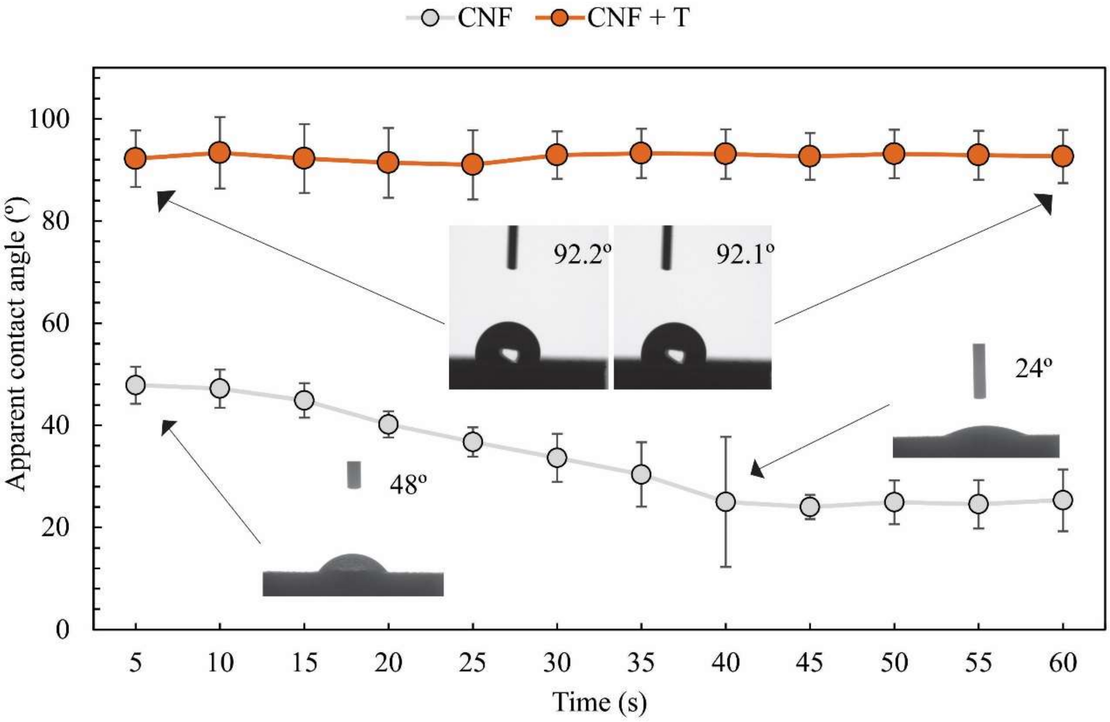

Fig. 5. Apparent contact angle of films with nanocellulose and tannin.

The incorporation of tannin renders the cellulosic film more hydrophobic and impairs the water absorption, since the apparent contact angle is always higher than 90° during the first minute of measurement. The hydrophobization of the surface can be due to two combined effects: (i) the chemical one of having aromatic substances like the polyphenols, limiting the contact with the cellulose and (ii) the physical one, which consists in “glue-like” effect decreasing the access of water between the cellulose nanofibers.

The high density of the film combined with the enhanced hydrophobicity, allow considering this material as a very effective physical barrier (Hubbe et al., 2017; Lavoine et al., 2012).

3.4.Thermogravimetric analysis

Regardless the water desorption, the thermal degradation of the pure cellulose film occurred in only one-step (Fig. 6). The mass loss related to the structural decomposition of the cellulose film took place at 340°C, with onset temperature at 250°C. From this temperature onward, dehydration of the cellulose happens and saturated carbonaceous structures are formed, then charcoal is produced (Lengowski et al., 2016; Wang et al., 2013).

The thermal degradation pathway observed for the pure tannin was completely different from the cellulose. The mass loss started at earlier temperature (150°C) and kept decreasing with low rate until 200°C, where another thermal degradation event occurred. It can be also observed that at higher temperature (>350°C) the tannin impart a certain degradation resistance due to the aromatic rearrangement (Tondi et al., 2008a; Tondi et al., 2008b). This thermal degradation pattern states the more complex chemical structure of the tannin when compared with cellulose.

However, the film prepared by adding tannin to nanocellulose in ~20% (CNF-T films) presented dominant thermal degradation characteristics from the nanocellulose net. In fact, as the CNF-T thermogram is not an overlapped result from the thermograms of the starting materials, therefore it is possible to state that CNF-T films are not just physical mixtures of the precursors.

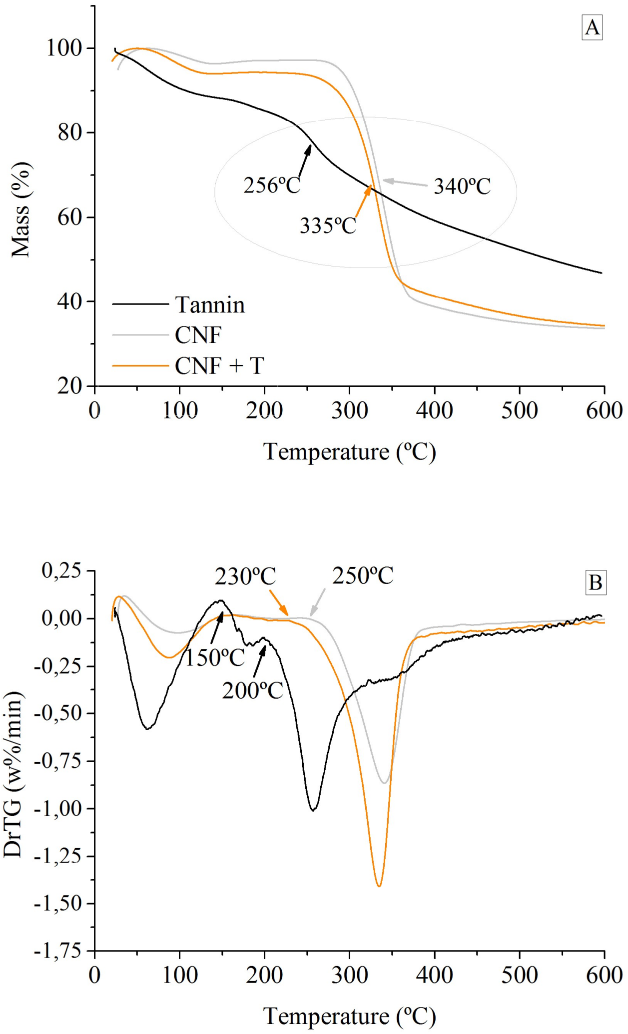

Fig. 6. Thermogravimetric curves (A) and their first derivative (B) of CNF, CNF-T films and tannin.

Tannin is highly water-soluble (Hemingway et al., 1989), as a result, during the high-shear milling process the tannin molecules could adsorb onto cellulose surfaces in a pseudo-molecular level. Higher thermal energy is required to decompose tannin in its polyphenolic branched structure, if compared to it adsorbed on a substrate in a pseudo-molecular level. In the first scenario, thermal energy is demanded to break the cohesive intermolecular forces and to separate the tannin macromolecules into small units for further decompose it. In the last, thermal energy is demanded only for decompose the tannin molecules since it is already separately adsorbed, on nanocellulose in this case. This justifies the lower thermal decomposition temperature observed for CNF-T film when compared with CNF films. It happened because the lower thermally stable tannin molecules induced the early degradation of the nanocellulose, which is the major component of the film. This suggest an intimate contact between tannin and nanocellulose so that the tannin only slightly anticipate the degradation of the nanocellulose fibers. This analysis confirms that the tannin molecules are deeply embedded in the cellulose net.

3.5.Antioxidant activity

The films of nanocellulose with and without tannin were measured for their antioxidant activity from 1 to 48 hours of water exposure and the concentration decay was registered by ORAC essay (Fig. 7). The water solution in which the CNF were dipped did not present any antioxidant property. Conversely, the formulation containing tannin presented a significant activity already after 1 hour (101.51 ± 2.55 μmol TE/g) with a maximum of antioxidant capacity after 8 hours (151.18 ± 3.44 μmol TE/g) and still very high values after 48 h (139.87 ± 2.60 μmol TE/g). These results suggest that the CNF-T release tannins in the solution immediately and continue to release them until 8-12 hours. Then, it slowly decreases its antioxidant activity and after 48 h it is still 17.7% higher than the antioxidant activity of Trolox. This property promotes the material for active packaging (López-de-Dicastillo et al., 2012; López de Dicastillo et al., 2011), especially when quick release of antioxidant should occur when the packaging enter in contact with water.

It is interesting to observe how the antioxidant activity decreases with a relatively contained slope. Figuring out the condition in which the tannin will be released continuously from the CNF-T, the antioxidant activity of the solution may be relatively high at the beginning and then, slowly decreasing when the tannin will be not free to escape from the cellulosic net. In principle, the presence of tailored amount of tannins will allow to apply this active packaging material for various purposes, which may vary from the food to the pharmaceutical industry (Klemm et al., 2011; López de Dicastillo et al., 2011; Zhou et al., 2016).

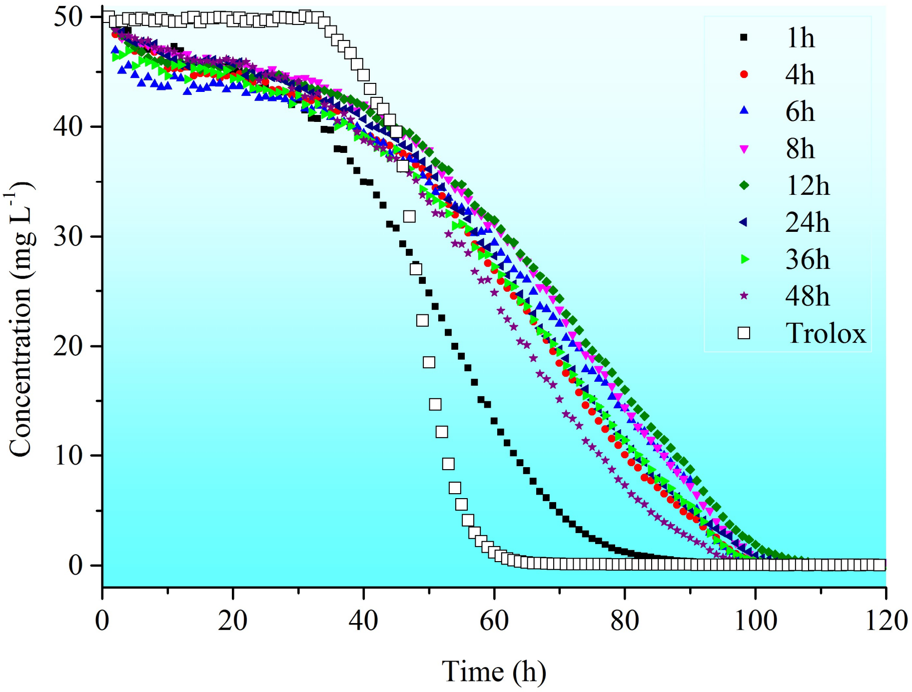

Fig. 7. Concentration decay of the antioxidant capacity over the time of tannin films obtained by ORAC assay.

3.6.Tensile strength & chemical resistance

The cellulose films CNF and CNF-T were exposed for seven days to different chemicals: solvents, alkali and acid and the mechanical resistance of the sample after drying and conditioning are reported in Fig. 8. Before any chemical test, the CNF-T resulted around 20% weaker than the tannin-free CNF film. This can be due to the presence of tannin which interfere with the optimization of the intermolecular hydrogen bonds between nanocellulosic fibers. However, the mechanical performance of the CNF-T films are comparable to that registered by other cellulosic-derived films (Olejar et al., 2014) and even higher than plastics (Magalhães et al., 2013) and glass fiber-reinforced polyethylene (Bajpai, 2017).

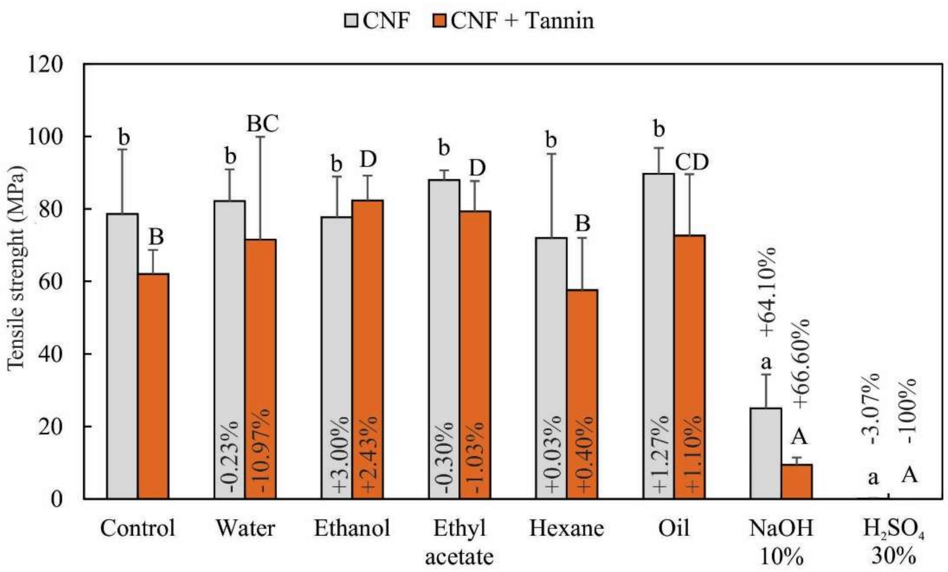

Fig.8. Tensile strength of films before and after chemical resistance tests. The same letters above the bars, separately for CNF and CNF-T films, indicate that the results are not statistically different according to LSD Fisher test at 5% significance. The values inside the bars indicate the mass loss (in %) of the film after the chemical resistance test.

The CNF and CNF-T films showed contained mass loss (or mass gain) for the majority of the solvents. The only significant exception was the material loss registered for the nanocellulose-tannin films when exposed to water. In this case, the mass loss of the CNF-T films was quantified as 10.97% after 7 days of dipping. Considering the weight loss due only to tannin, this value suggests that around 45% of the original tannin in the film still remains in the matrix after such a long exposure, which means that these films could still have some antioxidant activity. The tensile strength of the CNF-T resulted slightly enhanced, which means that the tannins do not participates in the mechanical property of the film and, hence, the active packaging material do not lose its mechanical performances after water dipping. The solvent exposure showed limited or no effect on the mechanical performances of the tannin-free films which only result slightly enhanced after the exposure to oil (but not significantly). Conversely, the tannin-containing films showed improved performances after dipping in ethanol and ethyl acetate even if no significant weight changes were registered.

This phenomenon can be explained by the increase of mobility of the flavonoids exposed to polar solvents: The tannin molecules become more mobile (but not enough to become soluble) and they arrange in a way that allows the optimization of the secondary forces (between cellulose and tannin fractions) during drying. The good chemical resistance observed against solvents was not repeated for alkali and acids: The exposure to alkali reduced the tensile strength of 68 and 84% for CNF and CNF-T respectively, while the exposure to sulfuric acid crumbled the films during the drying phase. Summarizing the films result highly resistant against solvent exposure, while they result dramatically degraded by alkali and acids. The only solvent that significantly interact with the tannin-containing film is water, which confirms the release of tannin also during weekly exposures.

The film containing tannin (CNF-T) are for their composition 100% natural and biodegradable. They have a dense and highly hydrophobic surface and therefore they are candidate to have good barrier properties. These films result thermally resistant until around 230°C and chemically resistant against organic solvents. When in contact with water, the films release around 55% of the original tannin in one week and the tannin released offers a good antioxidant activity for at least 48 hours.

These properties promote the material for a wide range of application in packaging technology: From the increase of the shelf-life of dry food like rise or pasta, to the conservation of fruit, vegetables and meat, but also for the external packages of oxidation-sensitive pharmaceuticals or cosmetics.

4. Conclusions

Nanocellulose films with around 20% of intimately connected tannin were produced. These new active packaging materials present high density and significantly improved hydrophobicity, which suggests also high barrier properties. In spite of the reduced mechanical performances, the CNF-T have shown very good thermal and solvent resistance.

Outstanding antioxidant activities were measured for the CNF-T. Already after 1 hour of dipping and during all the measured period of 48 hours the antioxidant activity resulted superior than that of a synthetic antioxidant used and in the first 8-12 hours the oxidation resistance was always increasing. The tannin-added nanocellulose films appears to be a very promising material for a completely natural active packaging for food and drugs.

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6. DISCUSSÃO

A utilização de matérias primas renováveis, sustentáveis e a partir de fontes naturais para elaboração de novos materiais aumentou significativamente nos últimos anos. Os principais motivos podem estar relacionados a consciência atual da população mundial por soluções ecológicas, além da tentativa de substituir materiais baseados na cadeia produtiva do petróleo e/ou materiais tóxicos. O desenvolvimento de atividades de inovações tecnológicas no Brasil, principalmente quando baseadas em recursos naturais presentes em abundância, devem e necessitam ser incentivadas. A biodiversidade em relação a fontes de possíveis novas moléculas e compostos é imensa, que dependendo do nível de inovação empregado podem gerar novos materiais.

Em termos gerais, a utilização de taninos condensáveis como matéria prima para obtenção de diversos produtos e subprodutos pode ser transpassado ao conceito de produção em biorrefinarias integradas, em que objetiva-se a eficiência, maximizando os benefícios e o lucro com a utilização de biomassa. Além disso, diversificar o uso de matérias primas e tecnologias de processamento, além de reduzir a dependência da produção de somente um produto, pode fornecer combinações que atendam às necessidades locais e também prioridades nacionais em maior escala.

No artigo 1 nós apresentamos uma introdução inicialmente sobre “bioresources” até encontrarmos o material principal dessa pesquisa – taninos condensáveis. A partir disso, como parte de um embasamento teórico-literário sobre nossa matéria prima, listamos alguns dos principais novos materiais que possam ou que estão sendo desenvolvidos utilizando taninos condensáveis e suas propriedades tecnológicas. Dessa lista de materiais, escolhemos para detalhamento três temas, (i) a purificação dos extratos tânicos, (ii) utilização do tanino como compatibilizante em compósitos plástico-madeira e (iii) confecção de filmes de nanocelulose e tanino para embalagens ativas.

A purificação ou fracionamento de materiais biológicos tem por objetivo obter frações com características específicas para aplicações como embalagens, alimentos, farmácia, medicina e nanomateriais em geral. Extratos de taninos e mais especificamente os de Acácia Mimosa, além de apresentar elevada quantidade de taninos condensáveis (~70-80%) também contém impurezas – hidrocolóides, ácidos orgânicos e açúcares. Nesse sentido, para otimizar e alcançar o máximo das propriedades dos taninos condensáveis, os extratos precisam ser purificados. No Artigo 2 nós apresentamos uma caracterização analítica de frações purificadas do extrato de tanino utilizando diferentes solventes orgânicos em um extrator Soxhlet. Esse método caracteriza-se por ser simples, sem a necessidade de equipamentos caros e nem recursos humanos específicos.

Os resultados apresentados no Artigo 2 mostraram que a purificação utilizando solventes com diferentes polaridades foi realizada com sucesso. Foram obtidas frações com baixo peso molecular, alto teor de fenólicos e antioxidantes, baixo teor de cinzas (fração acetato de etila) e também com elevado peso molecular e baixo teor de carboidratos (fração pentanol). Nós mostramos no mesmo artigo, que a utilização de um fracionamento sequencial com os mesmos solventes pode ser realmente interessante, já que com os solventes apolares no início da purificação (acetato de etila e hexano) é possível remover frações com característica de taninos hidrolisáveis e açúcares. Assim, com a sequência da purificação, obtemos diferentes frações de taninos condensáveis utilizando frações alcoólicas - polares, que podem ser aplicadas em finalidades distintas, como adesivos (maior peso molecular) e como antioxidantes para fármacos (menor peso molecular e maior reatividade).

Inicialmente então, nós purificamos nossa matéria prima principal a fim de obter um produto com características diferentes das apresentadas anteriormente. Nos artigos subsequentes da tese, tentamos utilizar a nossa matéria prima em produtos conhecidos ou elaborar novos produtos com conceitos atualizados na área florestal madeireira e/ou materiais. No Artigo 3 utilizamos o tanino como um compatibilizante sustentável na elaboração de compósitos plástico-madeira. Verificamos que a inserção do tanino, principalmente em compósitos confeccionados com partícula de pinus, propiciou resultados interessantes. O módulo de armazenamento medido com variação de temperatura apresentou nítido acréscimo para os compósitos com partícula de pinus e tanino, além de os mesmos compósitos terem apresentado menos espaços vazios visualizados pela microscopia por varredura eletrônica. Todos os compósitos confeccionados com tanino não apresentaram variação nas propriedades mecânicas de flexão, as quais normalmente são aumentadas com a utilização de compatibilizantes sintéticos. Dessa forma, é importante ressaltar dois pontos: (i) compatibilizantes naturais como lignina e agora o tanino utilizado pelo nosso grupo podem apresentar essas características, de não acréscimo de propriedades mecânicas específicas, e (ii) especificamente em nossa pesquisa, quando adicionamos 15% de tanino também reduzimos 15% de material lignocelulósico, o qual é normalmente responsável pela resistência mecânica analisada, sendo a perda ou não variação justificável. Dessa forma, a adição de um material não fibroso e a consequentemente não perda de resistência mecânica é muito interessante, e deve ser detalhada mais profundamente nas próximas pesquisas.

No Artigo 4 nós conseguimos com sucesso preparar filmes de nanocelulose e tanino utilizando uma nova técnica. A partir da característica polar dos extratos de tanino, sua inserção em uma solução com 2% de nanocelulose e 98% de água se torna um desafio, já que durante o processo de preparação do filme, sem uma ligação entre tanino e a nanocelulose, todo o tanino é filtrado juntamente com a água, formando um filme somente com a matriz lignocelulósica. Dessa forma, durante o processo de fabricação da nanocelulose, no moinho microprocessador, inserimos o tanino, com o objetivo de alcançar uma melhor ligação entre os materiais. Com essa metodologia, conseguimos a ligação de pelo menos 10% do tanino inicial na nanocelulose, comprovada pela análise morfológica e térmica, sendo o primeiro grupo de pesquisa no mundo a apresentar esse filme sem adição de adesivos ou agentes de ligação. A partir da caracterização desse material, verificamos uma elevada capacidade antioxidante quando o tanino foi adicionado, além de elevada propriedade mecânica e capacidade de barreira por meio das análises de molhabilidade, densidade e morfologia de superfície. Dessa forma, apresentamos uma embalagem ativa confeccionada 100% com material orgânico, sustentável e de fontes renováveis, a fim de substituir as tradicionais embalagens de plástico causadores de muitos problemas ambientais.

Nesse contexto, a partir do objetivo principal da tese de apresentar novas estratégias de valorização do extrato de taninos e, sabendo que para tal necessita-se conhecimento profundo de sua matéria prima, ressaltamos que os Artigos 1, 2, 3 e 4 cumprem o previamente proposto. Conseguimos detalhar um embasamento teórico, caracterizamos analiticamente o extrato de tanino e suas frações purificadas, além de termos elaborado e direcionado o tanino para dois novos campos de mercado – compósitos plástico-madeira e embalagens ativas.

7. CONSIDERAÇÕES FINAIS

Na pesquisa apresentada propomos a obtenção de novas estratégias para valorização do extrato de tanino obtido industrialmente. Materiais utilizando taninos são mundialmente conhecidos, sendo o extrato direcionado principalmente para áreas de curtimento de couro, adesivos e floculantes. Novos materiais utilizando taninos são e devem ser produzidos, levando em conta principalmente propriedades características dessa matéria prima, como por exemplo a capacidade antioxidante e antimicrobiana, de adesão e de ligações químicas com outros materiais.

A purificação do tanino utilizando diferentes solventes orgânicos foi realizada com sucesso. Tanto no método simples quanto no método sequencial, encontramos frações com diferentes rendimentos (variação de polaridade do solvente), teor de fenólicos, taninos condensados, cinzas, peso molecular e capacidade antioxidante. Assim, frações com menor peso molecular indicaram maior capacidade antioxidante, podendo ser usadas para finalidades como embalagens ativas, e fármacos. Frações com pelo molecular maior e as demais propriedades ainda elevadas podem ser facilmente implementadas como adesivos ou compatibilizantes.

Compósitos plástico-madeira com tanino como compatibilizante foram produzidos com sucesso. Esses produtos apresentaram características específicas, como maior hidrofobicidade e maior módulo de armazenamento até a temperatura de 75ºC, além de propriedades mecânicas de flexão e dureza similares aos compósitos padrão, mesmo com 15% menos material lignocelulósico. A partir desses resultados indica-se a utilização desses compósitos para aplicações externas, como decks e revestimentos. Indica-se testes nesse sentido a fim de avaliar a capacidade de proteção biológica e UV dos compósitos, além de variar proporções de tanino, material lignocelulósico e matriz plástica.

Utilizando uma inédita metodologia, conseguimos confeccionar filmes de nanocelulose e tanino sem utilização de qualquer adesivo, agente de acoplamento ou surfactante. Os filmes caracterizaram-se por apresentar elevada propriedade de barreira a líquidos, resistência a solventes químicos, alta resistência mecânica, além da significativa propriedade antioxidante. Essas propriedades potencializam a utilização desse filme como uma embalagem ativa, principalmente para o setor de alimentos e cosméticos. Indica-se a utilização desses resultados há elaboração de novos filmes, com diferentes concentrações de tanino e agentes de ligação.