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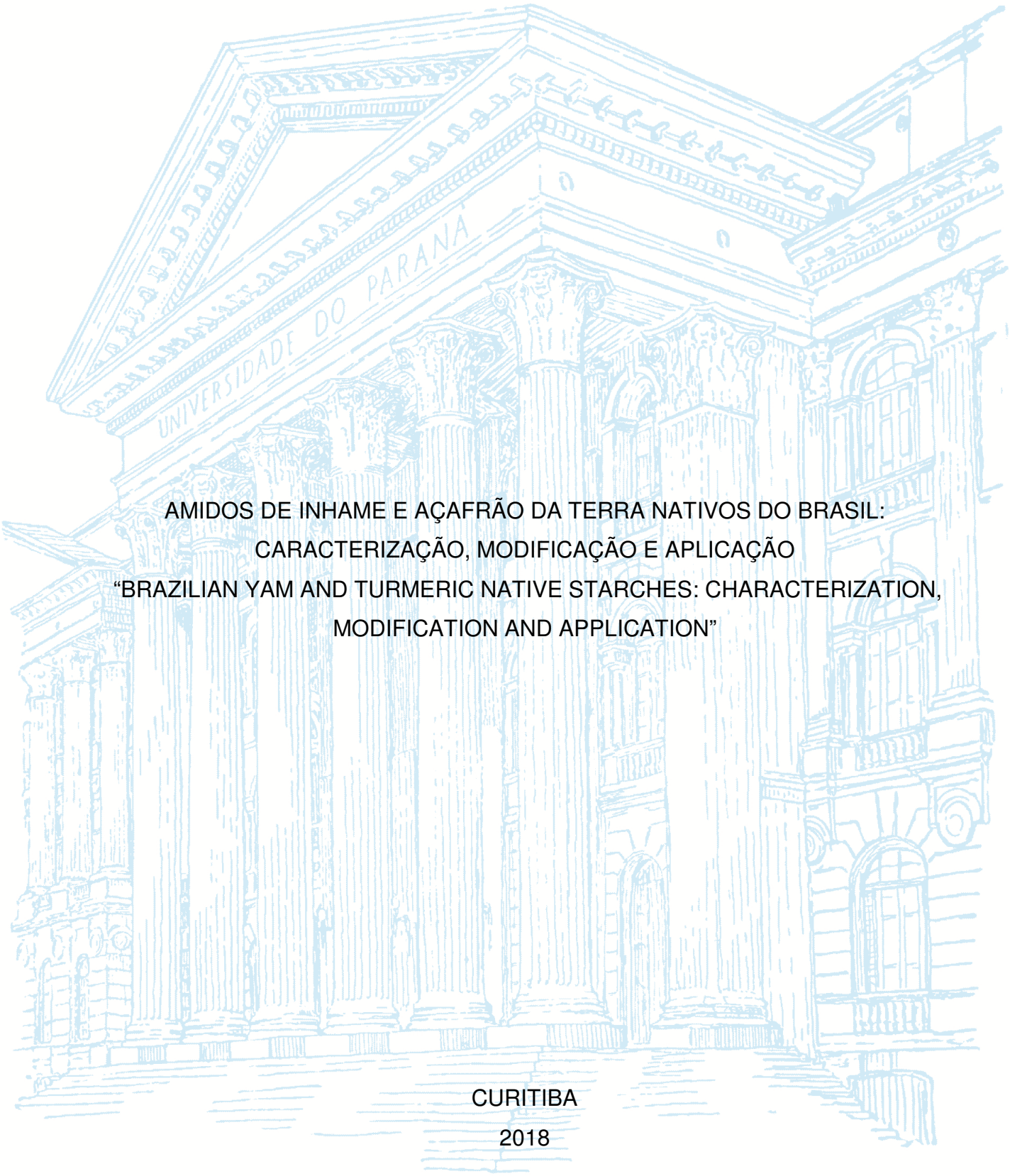

AMIDOS DE INHAME E AÇAFRÃO DA TERRA NATIVOS DO BRASIL: CARACTERIZAÇÃO, MODIFICAÇÃO E APLICAÇÃO

"BRAZILIAN YAM AND TURMERIC NATIVE STARCHES: CHARACTERIZATION, MODIFICATION AND APPLICATION"

CURITIBA

2018

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Orientadora: Profª. Drª. Rosemary Hoffmann Ribani
Coorientador: Prof. Dr. Marcelo Lazzarotto
Supervisora Internacional: Profª. Drª. Trust Beta

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ENGENHARIA DE ALIMENTOS da Universidade Federal do Paraná foram convocados para realizar a arguição da Tese de Doutorado de **POLYANNA SILVEIRA HORNUNG**, intitulada: **AMIDOS DE INHAME E AÇAFRÃO DA TERRA NATIVOS DO BRASIL: CARACTERIZAÇÃO, MODIFICAÇÃO E APLICAÇÃO**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

A outorga do título de Doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 21 de Maio de 2018.

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EGON SCHNITZLER(UEPG)

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RESUMO

A determinação das características dos amidos nativos é crucial para direcionar sua aplicação na indústria. As propriedades térmicas, reológicas e estruturais do amido nativo das espécies de Dioscoreaceas foram caracterizadas e comparadas com as propriedades dos amidos comerciais e apresentadas no CAPÍTULO 2. Os resultados de termogravimetria (TG/DTG) mostraram que o amido nativo da tuberosa *D. piperifolia* obteve a maior estabilidade à degradação entre amostras, exceto quanto ao amido de batata. Além disto, através das técnicas analíticas de calorimetria exploratória diferencial (DSC) e viscoamilografia (RVA) foi possível determinar que os amidos nativos obtiveram as maiores temperaturas de início de gelatinização (T_o – "*onset*") e maior tendência à retrogradação. Geralmente os amidos nativos não possuem as propriedades requeridas para suportar processos produtivos. Por este motivo, foi desenvolvido no CAPÍTULO 3 um método de modificação limpa "verde" empregando a mistura de diferentes espécies dos amidos de inhame estudados no CAPÍTULO 2, incluindo-se a cultivar *Dioscorea trífida*. Pela Calorimetria Exploratória Diferencial (DSC) e Viscoamilografia (RVA), verificou-se que o conteúdo de amilose do amido nativo de *Dioscorea sp.* influenciou nas propriedades de gelatinização e pasta das amostras após a mistura. Além disso, utilizando a análise de Microscopia Eletrônica de Varredura (MEV), foi possível sugerir uma correlação entre o tamanho dos grânulos de amido e seu grau relativo de cristalinidade, que foi calculado pelos difratogramas de raios X (XRD). No CAPÍTULO 4, um processo limpo "verde" para obtenção de embalagem biodegradável foi desenvolvido. Os filmes obtidos após o processo de mistura apresentaram baixo teor de umidade e alta transparência, alta solubilidade, características desejáveis para biodegradabilidade. Da mesma forma, a barreira à luz e a barreira de permeabilidade ao vapor de água foram aperfeiçoadas. Usando a calorimetria exploratória diferencial, observou-se que a temperatura de transição vítrea dos filmes diminuiu. A mistura de amido aprimorou as características mecânicas dos filmes em 200 % para resistência à tração e 232% para o alongamento à ruptura. O CAPÍTULO 5 apresenta o estudo da composição centesimal do amido nativo do açafrão da terra (*Curcuma longa* L.) a identificação e quantificação por HPLC-MS e "Folin-Ciocalteu" dos compostos fenólicos, da atividade antioxidante por DPPH, ABTS e ORAC do amido nativo e da embalagem obtida de *Curcuma longa* L. comparando com os resultados obtidos para o amido e filme de milho comercial. As propriedades mecânicas, térmica, de espessura, cor e superfície do filme obtido igualmente foram investigadas e comparadas. O HPLC-MS revelou oito compostos fenólicos principais no amido de *C. longa* e seis em seu filme. O amido e o filme de *C. longa* apresentaram alta capacidade antioxidante, 65 – 92 % (DPPH e ABTS). Além disso, o filme do amido de *C. longa* exibiu uma superfície de estrutura lisa e uma alta resistência à força de tração, mantendo sua elasticidade. A análise de cor apresentou a tendência à cor amarela do filme de açafrão que pode exercer excelente barreira à luz. Com base nos resultados obtidos, uma nova película de amido biodegradável e naturalmente bioativo foi desenvolvida aplicando-se o amido nativo da *Curcuma longa* L..

Palavras-chave: Dioscoreáceas, amido modificado, modificação limpa "verde", cúrcuma, filme amiláceo, filme bioativo.

ABSTRACT

The determination of the characteristics of the native starches is crucial to direct their application in the industry. The thermal, rheological and structural properties of the native starch of Dioscoreaceae species were characterized and compared with the commercial starch properties presented in CHAPTER 2. Thermogravimetric (TG / DTG) results showed that the native starch of *D. piperifolia* tuberose had the greater stability to degradation between samples, except for potato starch. In addition, by analytical techniques of differential scanning calorimetry (DSC) and viscoamilography (RVA) it was possible to determine that the native starches obtained the highest gelatinization (*To* - onset) temperatures and a tendency to retrograde. Generally, native starches do not have the properties required to support productive processes. For this reason, a "green" modification method was developed in CHAPTER 3 using the mixture of different species of the yam starches studied in CHAPTER 2, including *Dioscorea trífida*. By Differential Scanning Calorimetry (DSC) and Viscoamilography (RVA), it was verified that the amylose content of the native starch of *Dioscorea sp.* influenced the gelatinization and paste properties of the samples after mixing. In addition, using Scanning Electron Microscopy (SEM) analysis, it was possible to suggest a correlation between the size of the starch granules and their relative degree of crystallinity, which was calculated by the XRD diffractograms. In CHAPTER 4, a "green" process for obtaining biodegradable packaging was developed. The films obtained after the mixing process presented low moisture content and high transparency, high solubility, desirable characteristics for biodegradability. Likewise, the light barrier and the water vapor permeability barrier have been improved. Using differential scanning calorimetry, it was observed that the glass transition temperature of the films decreased. The starch mixture improved the mechanical characteristics of the films by 200% for tensile strength and 232% for elongation at break. In the present study, the centesimal composition of native saffron starch (*Curcuma longa* L.) was determined by HPLC-MS and Folin-Ciocalteu quantification of phenolic compounds, antioxidant activity by DPPH, ABTS and native starch and the package obtained from Curcuma longa L. comparing with the results obtained for starch and commercial corn film. The mechanical, thermal, thickness, color and surface properties of the obtained film were also investigated and compared. HPLC-MS revealed eight major phenolic compounds in *C. longa* starch and six in its film. Starch and *C. longa* film had high antioxidant capacity, 65 - 92% (DPPH and ABTS). In addition, the *C. longa* starch film exhibited a smooth structure surface and a high tensile strength, while maintaining its elasticity. The color analysis showed the tendency to yellow color of saffron film that can exert excellent barrier to light. Based on the results obtained, a new biodegradable and naturally bioactive starch film was developed by applying the native starch of *Curcuma longa* L..

Key-words: *Dioscoreaceas*, yam, modified starch, green process, turmeric, starchy films, bioactive film.

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1 INTRODUCTION

Present in vegetable tissues as a reserve carbohydrate of higher plants, starch is a neutral polysaccharide formed of D-glucose units linked by α-1,4 bonds. Starch has unique characteristics attributed to structural formations in small individual conglomerates known as granules. The starch granules are composed of the alternately configured amylose and amylopectin macromolecules (XIE et al., 2015). The amylose is essentially linear, and the structural units exhibit α-1,4 linkages. Amylopectin, on the other hand, is a highly branched molecule structured by α-1,4 short chains with α-1,6 branching points (LI et al., 2011). Due to its constitution, the starch possesses technological properties of interest; therefore, it is the most applied component in the manufacture of food, paper, textiles, and pharmaceuticals, among others (GALLANT et al., 1992; LI et al., 2011).

Brazil, as well as other tropical countries, presents a great variety of amylaceous species little studied like species of the family Dioscoreaceae (yam) or applied as the *Curcuma Longa* L. rhizome (JIANG et al., 2012, 2014; PERONI, 2003). In recent years, because of the restrictions imposed on the use of chemically modified starches, due to the possibility of residues in the final product, the food industry has been searching for new sources of starches and techniques for modifying them by physical means or by mixing different starches (GARCIA et al., 2014). The mixture of starches from different botanical sources is presented as an alternative to the methods of chemical modification to transform the properties (retrogradation and enthalpy of gelatinization, among others) of the starch as required by the industrial processes (HAGENIMANA; PU; DING, 2005; OJEDA, ELIASSON, 2001).

Tubers of the genus *Dioscorea* (yam) present great potential for the extraction of starch. According to Ramos et al., (2014), it has superior characteristics to starches derived from cassava and maize (RAMOS et al., 2014). In addition to being sources of starch, significant amounts of minerals, thiamine, riboflavin, niacin and ascorbic acid, important protein and lipid contents are found in yam tuberose (CASTRO et al., 2012; MOLLICA et al., 2013).

Popularly known as turmeric (*Curcuma longa* L.) has more than 100 catalogued species (VAN HUNG; VO, 2017). Brazil has impressive cultivation of turmeric in the centre-west that corresponds to 26% of the production of the state of

Goiás, demonstrating the potential to supply the demands of the internal market (BERNI et al., 2014). Its starch content is a residue that represents 78-85% of the initial matter, and it has no commercial value (NGUYEN et al., 2014). *C. longa* rhizome starch represents an attractive low-cost renewable base material for bioplastics (NGUYEN et al., 2014).

One of the technological potentialities of starches and starch mixtures is their use in the production of biofilms instead of films derived from petroleum products (BASTOS et al., 2009; BORDÎRLAU et al., 2012; WU; SUN, 2013; XIE et al., 2014). Starch is one of the most widely used biopolymers to produce biodegradable materials due to availability and low cost (HENRIQUE; CEREDA; SARMENTO, 2008).

Given the stated, this study characterized the properties of the native starches from *Dioscorea* (yams) and *Curcuma* (turmeric) species. Besides that, it was investigated the alterations caused by mixing modifications on the technological features of the yams (*D. sp.*; *D. piperifolia*; *D. trifida*) native starches and obtained starch films. Moreover, biodegradable and bioactive starch film from the native turmeric was developed and characterized.

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

To study the properties of the native and modified starches from yam tuberous species *Dioscorea alata* (white yam); *Dioscorea trífida* L. (purple yam); *Dioscorea piperifolia* Humb var. Wild. (air yam) and from the rhizome species *Curcuma Longa* L. (turmeric) applying them as biodegradable films.

2.2 SPECIFIC OBJECTIVES

a) To extract and to characterize the native starches from turmeric and yam cultivars by thermal, rheological, structural and colorimetric assays;

b) To modify the native yam starch by mixing the native starches;

c) To characterize the yam modified starches by thermal, rheological, structural and colorimetric assays;

d) To develop starch films from the native and modified by mixing yam starches;

e) To develop a natural bioactive starch film from the native turmeric starch;

f) To characterize the thermal, structural and mechanical properties of the turmeric and yams starch films;

g) To characterize, quantify and identify the phenolic compounds of the turmeric starch film and to investigate its antioxidant properties.

CHAPTER 1

1 LITERATURE REVIEW

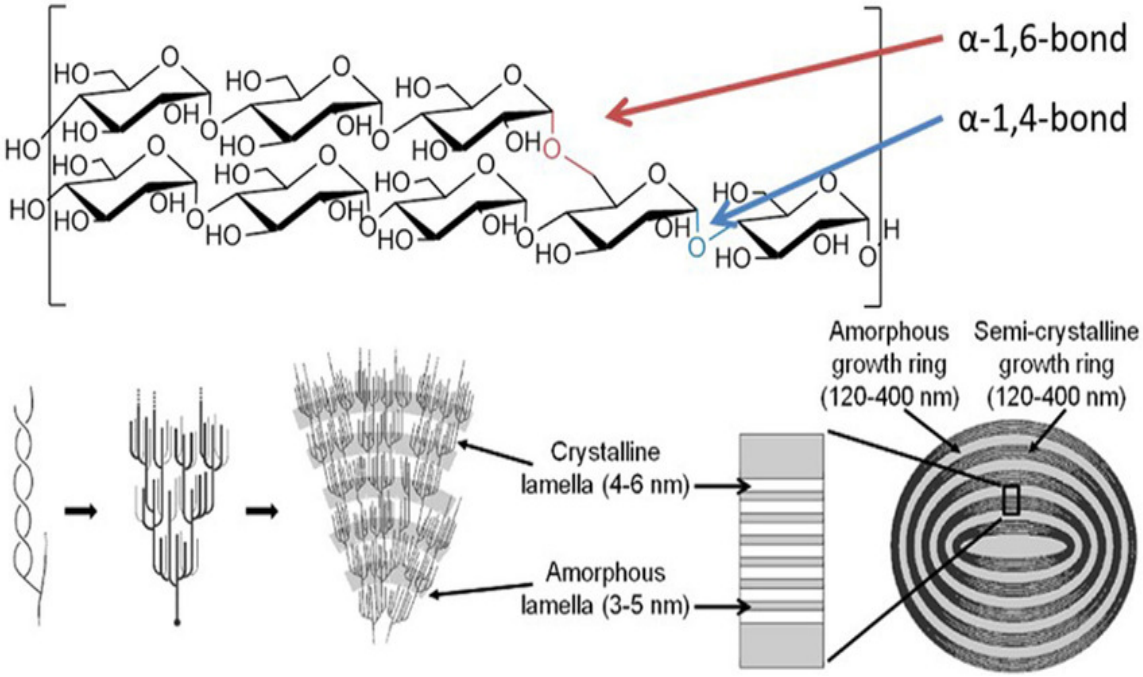
1.1 Starch – General Aspects

Starch is a neutral polysaccharide composed of α-1,4-linked D-glucose units. It is the main reserve carbohydrate of higher plants. Starch is present in all plant tissues such as fruits, seeds, roots and tubers. This raw material is capable of conferring technological attributes to the processed foods being the most applied element in the manufacture of foods. As for example, the use as a basis for the manufacture of extruded foods, snacks, breakfast cereals, biscuits, pasta and ready-to-eat foods.

Constituted by the amylose and amylopectin macromolecules, the starch is structured by small individual conglomerates called granules. Which are of different size and shapes. The sizes and shapes of the starch granules vary according to the botanical origin and stage of development of the plant. In general, starch granules have a diameter ranging from 1 to 100 μm, showing regular or irregular shapes (LIU, 2005; XIE et al., 2014). The morphology of the granules can be studied from optical birefringence, and X-ray diffraction analyzes, and can also be observed by electron microscopy.

The crystalline region of the starch granule is composed of the amylopectin chains, and are configured alternately to the amorphous regions (composed of amylose), as exemplified in Figure 1. Amylopectin chains are clustered in double helix bundles (DARDARDIN; SILVA, 2009). According to Billiaderis (1991), the granular structure of the starch is stabilized by the crystalline regions which also regulate the behaviour of the starch in the presence of water and may confer resistance to enzymatic or chemical modifications.

FIGURE 1. CRYSTALLINE AND AMORPHOUS REGIONS OF THE STARCH GRANULES



SOURCE: NEIL & FIELD (2015).

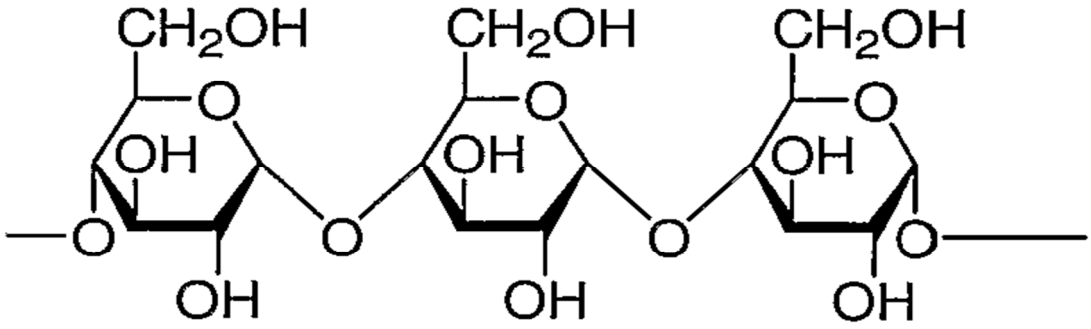
1.2 Amylose and Amylopectin

Amylose and amylopectin constitute 98-99% of the starch on a dry basis, differing according to the origin of the botanical species, and are directly linked to the functionalities of the starch pastes. Examples of starches with different amylose constitutions are "waxy" starches, which are less than 15% amylose; "normal" starch 20-35% amylose and "high amylose" starch containing more than 40% (MONTENSE et al., 2008; TESTER et al., 2004).

Amylose is a linear macromolecule (Figure 2) consisting of alpha-D-glucopyranose residues linked by α-1,4 glycosidic bonds and may contain from 350 to 1000 glucose units in its structure (LIU, 2005). On the average starch granules present amylose content around 20 to 30%, like corn starches 25 to 28%, cassava 17%, barley and rice varieties, whose starches are classified as (Waxy), have no amylose (WEBER; COLLARES-QUEIROZ; CHANG, 2009; XIE et al., 2014). These distinct contents of the amylose composition are responsible for the characteristics of the starch, for example, "normal" corn starches produce a consistent gel widely used in the manufacture of dehydrated soups and sauces requiring hot viscosity. However, they are not indicated in the processing of refrigerated foods, since it is highly

susceptible to syneresis (water exudation), a phenomenon that occurs in the retrogradation (PARKER; RING, 2001).

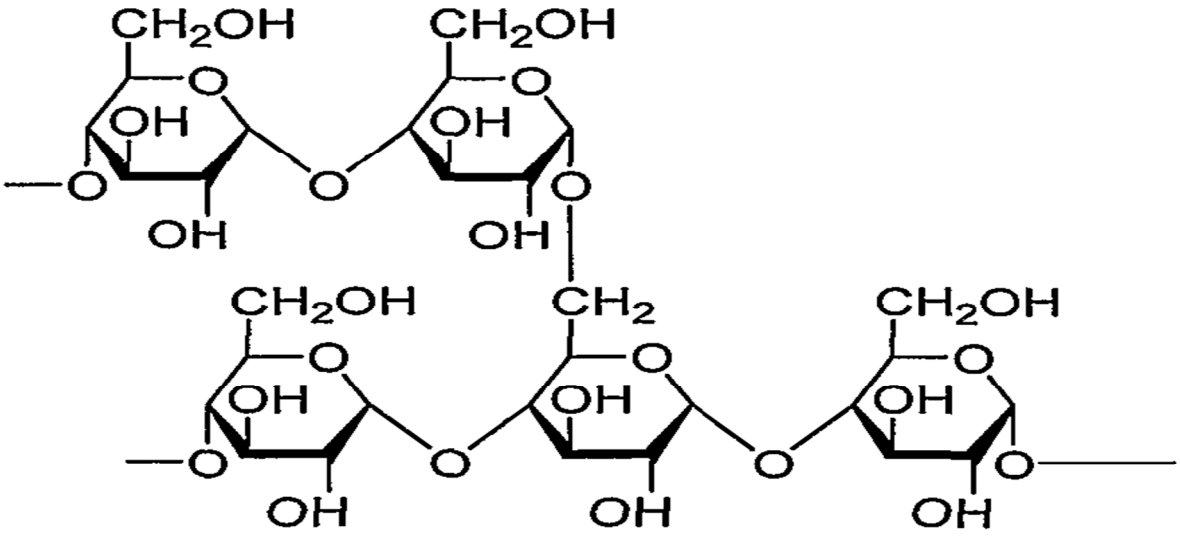
FIGURE 2. AMYLOSE MOLECULE STRUCTURE



SOURCE: ZAMBRANO; CAMARGO; TAVARES (2001).

Unlike amylose, amylopectin is a branched macromolecule (Figure 3) composed of α-D-glucopyranose units attached by α-1,4-glycosidic bonds and has α-1,6 branching points. Amylopectin, according to Cereda (2001), has a chain called the C chain which forms the main chain and carries the reducing group. Amylopectin has structures in double helices with a spherical shape and is very stable in the presence of water. Amylopectin can present from 10 to 500 thousand glucose units (ZAMBRANO; CAMARGO; TAVARES, 2001).

FIGURE 3. AMYLOPECTIN MOLECULE STRUCTURE



SOURCE: ZAMBRANO; CAMARGO; TAVARES (2001).

According to Wurzburg, (1986), amylopectin constitutes the starch in approximately 75%.

The amylose and amylopectin molecules can be distinguished by characteristics such as the order of magnitude of the molar mass, the degree of polymerization and other physicochemical properties (Table 1).

TABLE 1. AMYLOSE AND AMYLOPECTIN PHYSICO-CHEMICAL PROPERTIES.

Properties	**Amylopectin**	**Amylose**
Granular content (%)	65 – 85	15 – 35
Molar mass (Da)	107 – 108	103 – 105
Branches α-1,6 (%)	4 – 6	< 1
Polymerization degree	103 – 104	102 – 103
Chain length	3 – 50	3 - 1000

SOURCE: BALL; VAN DE WAL; VISSER (1998).

1.3 Thermal properties of the starch granules

1.3.1 Gelatinization

The insolubility of the starch in water is due to the strong hydrogen bonds between the amylose and amylopectin chains, despite the occurrence of swelling. Gelatinization occurs when the starch in the presence of water is heated. Heating induces amylose, the most soluble part, to dissociate and distribute out of the granule promoting gelatinization. The presences of D-glucose and hydroxyl groups, which are hydrophilic within the granule, facilitating swelling (PENG; ZHONGDONG; KENNEDY, 2007).

Generally, gelatinization occurs for most types of starch at a temperature between 60°C and 70°C (ELIASSON, GUDMUNDUND 2006, LAGARRIGUE et al., 2008). The amylose and amylopectin content, the shear rate, the time-temperature profile, pH and the presence of other constituents are factors that may interfere with the gelatinization process (MILES et al., 1985). Table 2 shows the peak gelatinization temperatures of the starches from some cultivars.

TABLE 2. TEMPERATURE INTERVALS OF PEAK GELATINIZATION OF STARCHES FROM SOME CULTIVARS OBTAINED BY DIFFERENTIAL EXPLORATORY CALORIMETRY (DSC).

Source	**Peak Temperature (*Tp*, ºC)**
Corn	62 – 72
Waxy Corn	63 – 72
Rice	61 – 77
Wheat	52 – 63
Cassava	58 – 70
Potato	56 – 66
“Pinhão”	50 - 57
Yam	66 - 75

SOURCE: BOBBIO; BOBBIO, 2003; COSTA ET AL., 2013; JIANG ET AL. (2012).

1.3.2 Retrogradation

After a period of storage or cooling, the gelatinized starch undergoes partial recrystallization of the amylose and amylopectin molecules. This process is known as retrogradation. Retrogradation directly interferes with the sensory quality (loss of moisture and consequent hardening of the final product) and nutritional (making the carbohydrate resistant to the digestive enzymes) of the product. The control of this phenomenon is of great technological and scientific importance (JAILLAIS et al., 2006; MUNHOZ, WEBER; CHANG, 2004; PATEL; SEETHARAMAN, 2010). As in the gelatinization process, certain conditions of storage, time, humidity and temperature are required for retrogradation to occur. To achieve retrogradation, water molecules are expelled from the gel network (sinerese), strong bonds are formed between the re-associated amylose molecules that are highly stable due to the hydrogen bonds formed (KOKSEL et al., 2008).

1.4 Starch Sources

The largest consumers of starch are the food industries; however, starch is also used in large quantities in the paper, chemical and textile processes. Due to the restrictions of the use of modified starches imposed mainly for the food industries,

there is a great interest in new sources of starches with characteristics that serve the consumer market (GARCIA et al., 2014).

In this context, tropical countries like Brazil present a great variety of amylaceous species that were not fully investigated. The characteristics of the starches from these sources could meet certain specificities of industrial processes. Therefore the study of the characterization of starches of new species becomes important to know the granular structure and physical-chemical properties that can present the desired behaviour for industrial processes. Research on amylose and amylopectin content branched chain size and distribution, contents of other components such as lipids, phospholipids, as well as grain size and shape, pulp properties and thermal properties determine the use of starches for the industrial processes (PERON, 2003).

The main commercial starch crops are corn, potato and cassava, but among other promising sources for starch production is the yams (MALI; GROSSMANN; YAMASHITA, 2010; PANKAJ et al., 2015; XIE et al. al., 2014). Corn, a plant of the species *Zea mays*, a term usually associated with seed is widely consumed as food (OLIVEIRA et al., 2010). An agricultural source of many applications corn is considered as gold since many others use it as a source of fuel. From this crop, the food industries obtain dextrin, oil, margarine, yeast, starch and others (CARDOSO et al., 2010). The corn grain varies in weight from 250-300 mg, consists of 72% starch (dry basis), 13.7% moisture, 0.05% protein, 0.12% lipid, 0.9% of fibers and by 27.8% of amylose and in addition, minerals in small quantities such as chlorine, calcium, sodium, iodine, manganese, iron, zinc, selenium copper among other constituents (PAES, 2006; COLLARES-QUEIROZ; CHANG, 2009). Due to the high starch concentration (61-78%) of the corn grain, it is the main raw material used by the starch industries (HU et al., 2014). The corn starch is an inexpensive, renewable raw material and meets the requirements of thermal stability and minimal interference with the fluidity and quality of the products (SABETZADEH; BAGHERI; MASOOMI, 2012).

The cultivation of cassava (*Manihot esculenta*, Crantz) originated in the American continent, possibly in Brazil, in the Amazon region bordering Venezuela. It was distributed throughout the world through Portuguese traders, before 1600, who took it to the African continent and then to East Africa and Asia (DEMIATE; KOTOVICZ, 2011).

Brazil occupies the third place after Nigeria and Thailand in the amount of cassava production. In the South American continent, Brazil is the largest producer followed by Paraguay and Colombia. The highest production volumes of cassava roots are concentrated in the states of Pará, Bahia and Paraná (BRAZILIAN INSTITUTE OF GEOGRAPHY AND STATISTICS, 2010). Table 3 shows the Brazilian states that extract in high quantity starch from the cassava root.

TABLE 3. NACIONAL PRODUCTION OF CASSAVA STARCH

States	**2017**	
	Production (t)	**% TOTAL**
Piauí	202.238	20 %
Pernambuco	178.820	17,7 %
Rio Grande do Norte	94.844	9,39 %
Santa Catarina	385.875	38,19 %
Amapá	148.650	14,71 %
TOTAL	**1.010.427**	**100 %**

SOURCE: CONAB (2017).

Some advantages related to other crops are presented by cassava, among them: easy propagation, high tolerance to long droughts, satisfactory yields even in low fertilized soils, potential resistance or tolerance to pests and diseases (DEMIATE; KOTOVICZ, 2011). Cassava, which contains high starch content (15-33%), is the basic source of energy of millions of people in the tropic regions all over the world. Other constituents are 60-70% water, 2.6% protein, 2.7% fiber and 0.3% lipid (HORNUNG et al., 2015, 2016).

The potato (*Solanum tuberosum* L.) is one of the most important and cultivated crops in the world (KHAN; ALI; HASNAIN, 2014). It is the fifth most economically important crop after sugar cane, corn, wheat and rice with a production of approximately 381 million tons in 2014 (FAOSTAT, 2017). Potato cultivation is important both for human consumption and for starch production. It is known to be highly nutritious, being a source of carbohydrates, high-quality proteins, vitamins (C, B6, and B3) and minerals (potassium, phosphorus and magnesium) (KITA et al., 2014 SIMKOVÁ et al., 2013). The potato cultivar is annually grown in almost every country in the world because of its natural adaptation to different soils and climates (MA et al., 2015). The European Union countries are responsible for the production of 1.8 million tonnes of potato starch leading the world production. Due to the low

cost of cultivation and extraction, availability and wide applicability, potato starch is considered a sustainable and economically crucial raw material (HONG et al., 2016). For the food industry, potato starch confers desirable properties on processed foods (KAUR; SINGH; SINGH, 2005). Unlike cereal starches, potato starch has unique features such as paste and gel formation with high viscosity, consistency and clarity (PARK et al., 2009). According to Noda et al. (2006), the high viscosity and transparency of the potato starch gel are due to the presence of phosphate. The phosphate content varies significantly, according to the cultivars and environmental factors.

1.5 The Yam (Dioscoreaceae)

It is imperative to study and research the physical, thermal, structural and pastes properties of new species of starchy plants for technological development. The starch of the tuberous species of the Dioscoreaceae family (yam) was very little studied and investigated, except for *D. opposita* and *D. alata* Linn species. Therefore, there is a vast field to be explored since more than 600 species have been identified (JIANG et al., 2012). The starch of *D. alata* Linn. exhibits technological features desired by industry such as high-temperature stability and low pH values. In addition to these characteristics, it has a similar flavour, texture and colour to corn starch and can be applied in the manufacture of fabrics, glue, syrups with high glucose content and bread products (DURANGO; SOARES; ANDRADE, 2009).

In Brazil, for a long time, the popular names of y “inhame” (yam in English) and “cará” have been causing great confusion. In the States of Paraíba and Pernambuco, it is common to name “inhame” the species of Dioscorea that produce large and small tubers. By the common name of yam are several known species of plants belonging to the family of Aráceas, but in other countries, even of the Americas, it is used for several species of the Dioscoreaceas family, instead of “cará” (SANTOS et al., 2007). In some regions of the country they also erroneously call yam the rhizome known as "taro" (*Colocasia esculenta*), confusing technicians, producers and consumers (LEONEL, CEREDA, 2002). In fact, many of the technical information, such as production and cost statistics, were also mixed. To overcome the confusion of the misnomer of popular names of the *Dioscorea* species, during the First National Symposium on Yam and “Cará” Cultures, the General Assembly proposed and

approved the denomination "inhame" or yam in English, for all species of *Dioscorea*, standardizing the Brazilian terms with the international denomination (LEONEL, CEREDA, 2002, SANTOS et al., 2007).

The tuber cultivars of the Dioscoreaceae family comprise in more than 600 species of which about 50 species are commercially grown worldwide for food consumption (CASTRO et al., 2012; FALADE; AYETIGBO, 2015; JIANG et al., 2014; NASCIMENTO et al., 2015; RAMOS et al., 2014). The yams originated in the African and Asian continents, and a large number of species are cultivated in Brazil (RAMOS et al., 2014). Thus, a diverse group of yams is found in Brazil, and these are consumed mainly by indigenous populations (RAMOS et al., 2014). Globally, the largest producer is Nigeria, which cultivates an area of approximately 3 million hectares, representing 67% of the world's tuber production (FALADE, AYETIGBO, 2015, FAO, 2009). The tubers of the species *D. esculenta* and *Dioscorea alata* are native to Central India; the *D. bulbifera* L. species originates from the Indo-Malayan region, and *D. trifida* L. is described in the literature in Central and South America. According to Caúper (2006), the species *D. trifida* is native and geographically distributed in the Amazon. It is estimated that in Brazil there are 130 species of *Dioscorea* that are present in all regions of the country (KIRIZAWA et al., 2010).

In China, different varieties of *Dioscorea* are of traditional therapeutic use in modern medicine and are consumed as the functional food of high economic value (JIANG et al., 2013, 2014). According to Hurtado et al. (1997), the yams spread in regions where the tropical climate predominates, and the plant has high adaptability, as it resists intense heat, long periods of drought and has a long shelf life in the post-harvest period. The tuber is used for both raw consumption and for flour and starch production (RAMOS et al., 2014). Matossian (1979) stated that the flour obtained from yam is an economically profitable substitute for wheat flour for the production of bread.

In addition to being an economically profitable crop for Africa, West and Southwest Asia, South America and India, yam is a potential source of starch for industrial applications (FALADE; AYETIGBO, 2015). Yam starch may substitute cassava and maize starch having the same properties regarding flavour, texture and colour (DURANGO, SOARES, ANDRADE, 2009; RAMOS et al., 2014; ZÁRETE et al., 1998). The species of tubers belonging to the genus *Dioscorea* are constituted by starch from 60 to 85% in dry basis, of these, 17 to 28% of the starch content is

composed of amylose (classified as "normal starch") (JAYAKODY et al., 2007, OKUNLOLA, ODEKU, 2011). Starch from yam species is promising for the development of edible films because of their high amylose content, forming resistant films (MALI; GROSSMANN; YAMASHITA, 2010).

1.5.1 *Dioscorea* Species Botanical Characteristics

Monocotyledonous, herbaceous, dioecious plants that are born in different male and female inflorescences. The yam is a tree-climber belonging to the *Dioscorea* genus of the Discoreaceae family (SANTOS et al., 2006). In Brazil, its flowering is rare, and the fruits are dehiscent capsules, and its pollination is entomophilic (bees, wasps and flies) (MONTEIRO; PERESSIN, 1993).

Widely composed of several species, the *Dioscorea* genus can be taxonomically divided as shown in Table 4.

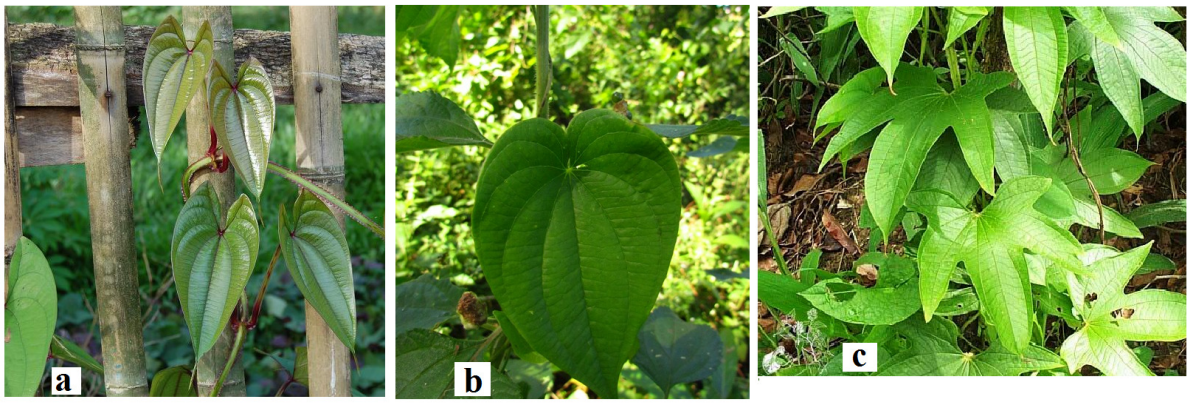
TABLE 4. TAXONOMIC CLASSIFICATION OF SOME SPECIES OF THE *DIOSCOREA* GENUS.

Species	**Taxonomic Classification**	**Main characteristic**
Dioscorea alata; Dioscorea rodundata; Dioscorea cayenensis	Enantrophyllum	Stems wind up clockwise.
Dioscorea esculenta	Combilium	The stems of these three classes are wound counterclockwise.
Dioscorea piperifolia	Osophyton	
Disocorea trífida L.	Macrogynodium	

SOURCE: BRESSAN ET AL., (2011).

In Figure 4, it is possible to observe the different characteristics presented by the leaves of the species *Dioscorea alata*, *Dioscorea piperifolia* Humb. var. Wild and *Dioscorea Trífida* L..

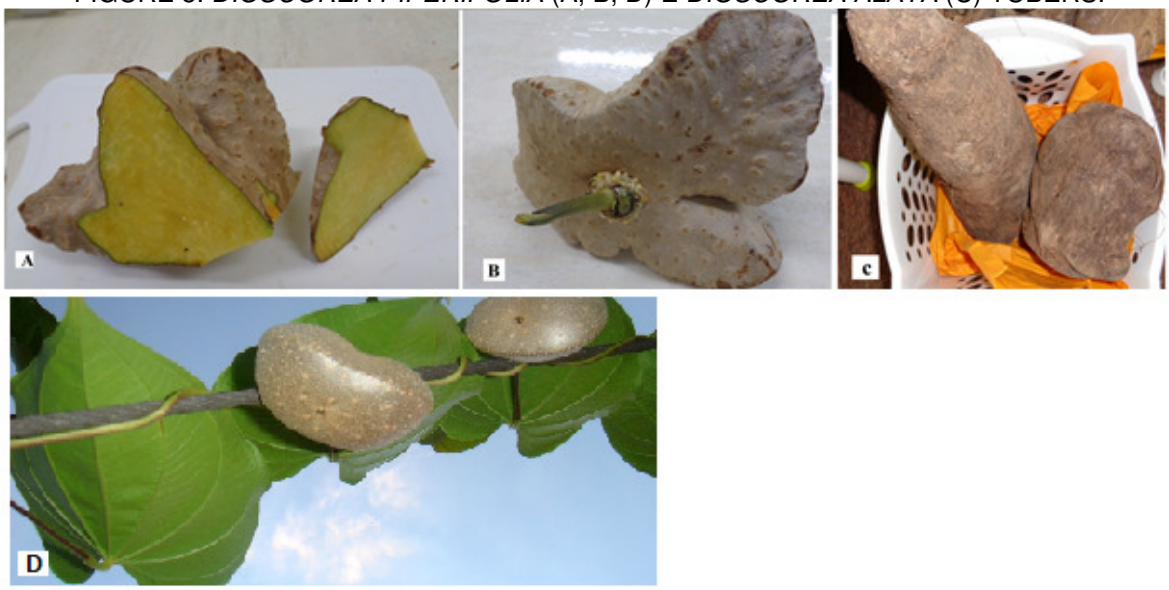
FIGURE 4. LEAVES CHARACTERISTICS OF *DIOSCOREA ALATA* (A), *DIOSCOREA PIPERIFOLIA* (B) AND *DIOSCOREA TRÍFIDA* L. (C).



SOURCE: The author (2018).

The species *Dioscorea alata* develops very well in regions where winter is prolonged. This species presents a square, winged, green stem with leaves of different sizes (Figure 5c) and cylindrical tubers of a variable size weighing between 5 and 10 kg (Figure 5, c) (PEIXOTO NETO et al., 2000). Yams from this species are considered to be very nutritious and easy to digest, and their flour is widely used in the production of "yam bread," which constitutes a staple food in several regions, mainly in the North, Northeast and Central West regions of Brazil (MOURA, 2006). According to Correa (1978), the introduction of this species in Brazil occurred in the colonial period probably brought by the slaves.

FIGURE 5. *DIOSCOREA PIPERIFOLIA* (A, B, D) E *DIOSCOREA ALATA* (C) TUBERS.



SOURCE: The author (2018).

Likewise, *Dioscorea piperifolia* Humb var. Wild. or air yam is a tree-climber, distinguished by being a robust plant that generates aerial tubers (Figure 5, D). Its stem is herbaceous, cylindrical, striated, folds counterclockwise with alternating leaves of 12 to 18 cm in length and 10 to 15 cm in width. The tuber of this species is considered in the literature as a fruit, described as an oblong capsule and each lobe contains winged seeds in the lower part, is very nutritious and tasty and the tubers can measure 10 cm in length. In the popular medicine of Brazil, the tubers are widely used as diuretics and as a cataplasm in furuncles (CORREA, 1978). These tubers began to be disseminated with the increase in the technological properties in regions of subtropical climate in the 80's (WOSIACKI et al., 2002).

Dioscorea trífida L. popularly known as purple yam, dark purple yam or pink yam among others, is also a tree-climber, has smooth stems, winged-striated at the bottom and angular-compressed at the top, curling in the same way that the stem of the air yam, counterclockwise; its tubers are 15 to 20 cm in length with purple starch (purple yam - Figure 6, a and b), pink or dark purple. This species in Brazil propagates in areas of tropical forest, in savannas of the Central region, in the rupestrian fields of the Serra do Espinhaço in Minas Gerais and the woods adjacent to these regions (NASCIMENTO et al., 2015). This culture has been maintained by traditional rural communities in the Ribeira Valley in São Paulo (BRESSAN et al., 2011), in Baixada Cuiabana in Mato Grosso (FERREIRA et al., 2010), in Manaus and surroundings, and in Belém do Pará (NASCIMENTO et al., 2015). The region of greatest cultivation of this species is the North region of Brazil (PEIXOTO NETO et al., 2000). *D. trífida* economically is considered one of the most important *Dioscorea* species (MOLLICA et al., 2013). In the Amazon, this tuber is consumed roasted, cooked in the form of a puree, as an ingredient for soups and stews, and in the formulation of flour for the production of cakes, pies and porridge. However, this species has been little studied scientifically; little is known about its nutritional potential, industrial use, conservation, characterization, uses as natural colouring and functional ingredient among others (TEIXEIRA, 2011).

FIGURE 6. *DIOSCOREA TRÍFIDA* TUBERS – PURPLE YAM (A, B).

SOURCE: The author (2018).

1.6 The Turmeric (*Curcuma longa* L.)

Popularly known as turmeric (*Curcuma longa* L.), this rhizome (Figure 7) originates in South Asia and has more than 100 catalogued species (VAN HUNG; VO, 2017). *Curcuma* belongs to the Zingiberaceae family genus, and it has been extensively investigated as a medicinal and pigment source. The Curcuma rhizome is widely cultivated and easily obtained: India is the main producer and consumer (ANUBALA; SEKAR; NAGAIAH, 2014). India harvested about 1,130 tons of fresh rhizomes or 80% of the world production market in 2017 (KHARIF, 2017). Brazil has impressive cultivation of turmeric in the centre-west that corresponds to 26% of the production of the state of Goiás, demonstrating the potential to supply the demands of the internal market (BERNI et al., 2014).

FIGURE 7. TURMERIC (*CURCUMA LONGA* L.) RHIZOMES.

SOURCE: The author (2018).

Moreover, its cultivation stimulates the economic growth of rural families in the northeast region of Brazil (BERNI et al., 2014). The primary use of the powdered rhizome of Curcuma is to extract oleoresin for pigments known as curcuminoids (curcumin; desmethoxycurcumin and bisdemethoxycurcumin) (BERNI et al., 2014; MANIGLIA et al., 2014; MUSSO; SALGADO; MAURI, 2016). India has 78% of the global turmeric market, and pharmaceutical applications account for over 52%. The quest for natural colourants in food and beverages has increased the demand for *Curcuma longa* L. (GLOBAL MARKET INSIGHTS, 2016). According to Van Hung and Vo (VAN HUNG; VO, 2017), the pigment only accounts for 2-8% of the powdered rhizome: its starch content is a residue that represents 78-85% of the initial matter, and it has no commercial value (NGUYEN et al., 2014). *C. longa* rhizome starch constitutes an attractive low-cost renewable base material for bioplastics (NGUYEN et al., 2014). Apart from its important phenolic compounds, which impart antioxidant, antibacterial and anticancer properties, another advantage of *C. longa* rhizome starch about developing starch films is its apparent amylose content of 48% (ANUBALA; SEKAR; NAGAIAH, 2014; VAN HUNG; VO, 2017).

1.7 Starch Modification Processes

The modifications applied to the native starch generate starch products with specific properties for their use in several processes increasing their valorization and their use (SANTOS, 2012).

According to the Ministry of Health (BRASIL, 1997), chemically modified starches are not considered as food additives and should be mentioned in the list of ingredients as modified starches.

Many types of chemical modifications have been applied to starch from different plant sources. The chemical modifications can cause structural alterations and the introduction of new functional groups, thus affecting the physicochemical properties of the starches, thereby increasing their use in the industry and providing starch products with properties necessary for specific uses (SANDHU et al. 2008).

There are several ways to classify starch modifications. One separates the modifications between physical, chemical, enzymatic or combined, another between degradative and non-degradative reactions. The degradative reactions alter the physical and chemical structure of the starch so that its properties are no longer

recognized. In non-degrading reactions the appearance and even some characteristics of the physical and chemical structure of the starch are maintained, then it may be difficult to recognize whether or not the starch was modified. Physical process modifications include the use of heat, radiation and shear (CEREDA et al., 2003).

Some factors such as starch composition, concentration and type of reagent, and reaction conditions may affect the reactivity of the starch during chemical modifications. The heterogeneity of the granules within a single source of starch may also affect the extent of the modification. The changes in the physical-chemical, morphological, thermal and rheological properties of the starches after the modification are the basis for the understanding of the efficiency of the starch modification process in industrial scale (SINGH et al., 2007).

According to Bemiller (1997), the reasons that lead to the modification of the native starch are: to modify the characteristics of cooking (gelatinization); decrease backwardness; reduce the tendency of pastes to form gels; increase the stability of pastes to cooling and freezing; increase transparency and improve the texture of pastes or gels; improve filmmaking; increase tack; add hydrophobic groups and introduce emulsifying power.

1.7.1 Alkaline Modification

Alkaline reagents such as sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3), for example, are widely used in the production of modified starches and by-products with different characteristics from native starches (LAI et al., 2002). Studies have been carried out on the effect of alkaline treatment, but the effect of the alkali has not been extensively studied when compared to the effects of acid and enzymatic hydrolysis of starch (MONDRAGON et al., 2004).

According to Karim et al., (2008) during the alkaline treatment the starch granule can change its microstructure and physicochemical properties. Such changes vary depending on the reagent used, the concentration, the time and the type of starch used also plays a significant role which affects the paste properties, especially the peak viscosity, breakdown, and degree of gelatinization. The alkaline treatment appears to preferentially attack the amorphous region of the granules being limited to amylopectin, leaving the crystalline part intact.

1.7.2 Acid Modification

The acid-modified starch suspension process is widely used in starch processing industries. The starch is suspended in dilute acid solution and maintained at the temperature ranging from ambient to below the gelatinization temperature. The suspension is stirred until the inherent viscosity of the starch is reduced to the desired value. The suspension is then neutralized with sodium hydroxide or sodium carbonate, washed and dried. The type of acid depends on the desired characteristics. After decanting, washing and drying, the modified starch hardly differs in appearance from the untreated starch. The variables of the type and acid concentration, temperature, starch concentration and reaction time should be selected depending on the process and the desired properties of the starch (MOORTHY, 2002).

During the acid treatment, amylopectin is preferentially and more rapidly degraded than amylose, with the action of the acid concentration in the amorphous region. As a result, there is a relative increase of amylose as a linear fraction (RIBEIRO; SERAVALLI, 2004). The main characteristic of acid-modified starches is the low hot viscosity. Pastes with low viscosity can be obtained during cooking and firm gels during cooling (CEREDA et al., 2003). Acid degradation involves mainly the breaking of the starch molecules into low molecular weight fragments. It increases the number of linear molecules smaller than the amylose molecules of native starch, altering its physicochemical properties, but leaving intact the crystalline structure of the granule. The result is the increase in the gelation tendency of the starch solution and gel formation under cooling and resting (SANDHU et al., 2008).

Studying the structure of the starch modified by acid hydrolysis is one way of improving understanding of the arrangement of amylose and amylopectin within the starch granule. Acid-resistant waste structures provide information about the nature of the crystalline region of the granule, which corresponds to the amylopectin cluster model (SRICHUWONG et al., 2005). Differences in the rate and degree of acid hydrolysis of granular starch have been attributed to differences in granular size, extent and type of interactions within the granule chain (e.g. degree and type of crystallinity) and starch composition (content of amylose and degree of phosphorylation) (HOOVER, 2000). The amylose content of the granules appears to

be one of the factors involved in the starch resistance by acid hydrolysis (KANG et al., 1997).

The acid modification has been used to improve the physicochemical properties of starch, particularly in food industries. The acid modification allows the starch to be used in a higher concentration of solids for rapid gelling, producing gums or jellies with better texture and flexibility (OGUNSANWO et al., 2005).

1.7.3 Oxidative Modification

Oxidation modification is produced by the reaction of starch with a specific amount of reagent at controlled pH and temperature. The starch can be oxidized by numerous agents such as ultraviolet light, sodium hypochlorite, calcium, ammonium persulfate, potassium permanganate, hydrogen peroxide, peracetic acid, sodium hydrochloride, perborates and hypochloric acid (ZHANG et al., 2007).

During the oxidation, the D - glycan α - 1,4 and D - glycan α - 1,6 bonds break. Since α-1,4 bonding is responsible for the binding of glucose monomers, oxidation causes depolymerization, and at the same time carbonyl (C = O) and carboxylic groups (COOH) may be included, which inhibit retrogradation (SOROKIN et al., 2004). The carboxylic groups, as they are larger than the hydroxyl groups, increasing the space between the amylose chains, preventing them from approaching enough to retrograde (DIAS et al., 2007).

Depending on the oxidizing agent and the reaction conditions, carboxyl and carbonyl groups can be generated (WANG et al., 2007). The importance of each type of reaction varies regarding the properties of the final product, it could have a higher number of hydroxyl groups at carbons 2, 3 and 6 (C-2, C-3 and C-6), compared to carbons 1 and 4 (C-1 and C-4). In this way, oxidation probably predominates in these sites, with a small amount occurring at the reducing and non-reducing terminals, C-1 and C-4, respectively (KUAKPETOON; WANG, 2008).

According to Takizawa et al. (2004) samples of oxidized modified starches have a high reducing power compared to the respective native starches. Glucose polymers were fragmented by oxidative treatment with reduction of molecular weight and exposure of a high number of reducing terminals. The efficiency of the oxidation can be affected by several factors such as pH, temperature, reagent concentration,

molecular structure and starch organization (SANCHEZ-RIVERA et al., 2005; SANDHU et al., 2008).

The oxidation of the starch produces a clear, fluid and adhesive paste which does not form a rigid gel upon cooling but retains its adhesive nature and fluidity. Films formed from oxidized starch pastes are strong, rigid and resistant in contrast to the weak and few resistant films of the acid-modified starch pastes (CEREDA et al., 2003). Oxidized starches are widely used in the paper, textile and food industries. The uses of oxidized starches in the food industry have increased because of its low viscosity, high stability, clarity, film formation and water retention property, and have been used as food coatings, binders in confectionery and as emulsifiers (KUAKPETOON; WANG, 2001).

1.7.4 Starch Mixture

In many applications, the properties of a native starch are not ideal, and therefore the starch is often chemically modified with the aim of improving its performance, especially in the industry. Nowadays, people seek to consume increasingly natural foods, avoiding chemical treatments as much as possible. It is of interest, especially in the industry, to find new ways of improving the properties of native starches without the use of chemicals. An alternative to chemical modification is the blending of different starches. According to Hagenimana, Pu and Ding (2005) and Ortega-Ojeda and Eliasson (2001) the mixture of starches from various botanical sources may show a reduction of the retrogradation, with each starch contributing to the enthalpy of gelatinization based on the ratio.

Structural modifications by physical or chemical means are often used to obtain desirable industrial starch utilization properties. Physical changes are preferred to chemical modifications, since they are simpler, safer and generally cost less (PARK et al., 2009).

According to Elgadir et al., (2012), the starch mixture is an old technique, widely used, artisanal and industrially. In the production of rice granules, for example, where corn starch and sago are mixed with rice flour, in addition to the reduction of costs, the use of these mixtures is due to the sensorial attributes conferred on the products, which are often not obtained using only one of the starches. When starches are mixed, changes in paste characteristics, texture and retrogradation occur without

changing the native starch structure. Starch molecules from different botanical sources can interact and thus produce attributes unique to the mixtures. Moreover, mixtures of starches generally exhibit unique viscoelastic properties which also depend on the combination of the mixed starches, the intrinsic properties of each starch, the relative size of the granules (PARK et al., 2009; ZHANG et al., 2011). Elgadir et al., (2012), reported that the characteristics of the blends are close to those of the modified starches, and may be due to the interactions between the solubilized parts of one starch with the granules of the other.

1.8 Starch films

Currently, there is a great interest in developing scientific and technological processes, generating products with high added value, with low environmental impact. In this way, the search for renewable raw materials and the sustainable use of natural resources and the consumption of natural products is increased. The use of packaging is an important factor for the preservation and intensification of the useful life of the food. The packaging reduces the diffusion of gases, the permeability to humidity, luminosity, among others, providing prolonged life and quality to the food products (CHEN; LIU; ZHANG, 2014; EÇA; SARTORI; MENEGALLI, 2014; MALI; GROSSMANN; YAMASHITA, 2010). According to the Ministry of the Environment (MMA), conventional packaging, derived from petroleum, is responsible for the generation of around 25 thousand tons of garbage per day in Brazil (BRAZIL, 2017).

Several countries recognize the serious problem that conventional packaging generates and are concentrating efforts on the research and development of ecologically sustainable alternatives. A solution is the development of biodegradable packages derived from renewable sources such as starch, proteins, alginates, pectin, cellulose derivatives and other polysaccharides (FARIAS et al., 2012). Starch is one of the most widely used biopolymers to produce biodegradable materials because of its low cost and availability (ARGÜELLO-GARCÍA et al., 2014). The gelatinized starch, when cooled, forms a thin film-like that is resistant and transparent (PAULI et al., 2011).

The application of starch in the production of films and coatings is based on the chemical, physical and functional properties of the amylose, because its linear structure can form gels and films (SKURTYS et al., 2001). The amylose molecules in

solution tend to be oriented parallel, approaching and forming hydrogen bonds between hydroxyls of adjacent polymers (MALI; GROSSMANN; YAMASHITA, 2010). In order to obtain a thermoplastic material based on starch, it is necessary to destroy the semi-crystalline granular structure (amylopectin) giving rise to a homogeneous and amorphous polymer matrix by means of gelatinization and the melting of the starch granules (HENRIQUE; CEREDA; SARMENTO, 2008, MALI, GROSSMANN, YAMASHITA, 2010).

The microstructure and the properties of the films and coatings of starch depend on the type of material used for their production (ARGÜELLO-GARCÍA et al., 2014; JIMÉNEZ et al., 2012). When produced on a laboratory scale, the films and coatings are formed after the solubilization of the starch in a solvent, generating the film-forming solution, the technique is known as casting. The solution is applied on a carrier and dried, and by evaporation of the solvent, the film is formed (MALI; GROSSMANN; YAMASHITA, 2010). Edible films and toppings can be made from any starch source containing amylose (KRAMER, 2009).

According to Rocha et al., (2014) it has been proven that the films produced from starch present an effective barrier to gases (CO_2 and O_2), but they have a high water vapour permeability due to the hydrophilic character of the polymers. An alternative to improve this property is the preparation of composite films, combining plasticizing materials, different sources of starches, the addition of oils, proteins and cellulosic materials. The plasticizers most used in films and coatings of starch are polyols such as glycerol and sorbitol, which provide mechanical improvement. When added to films, plasticizers reduce the interactions between adjacent molecules by reducing the hydrogen bonds between the polymer molecules making the film more flexible, therefore less brittle (AL-HASSAN; NORZIAH, 2012; SKURTYS et al., 2001; XIE et al. 2013, 2014).

1.9 Starch Characterization Analysis

Depending on the botanical source, applied modification, the starch can be used in the food industry to provide texture, serve as a thickener, and protect food during processing, among other functions. Thus, its application is directly linked to the functional properties (gelling, retrogradation, paste clarity and features) pertinent to its plant sources, such as granule size and shape, relative crystallinity, thermal

stability to degradation, amylose and amylopectin content. To investigate these properties, it is used standardized techniques such as Thermogravimetric Analysis (TG), Differential Scanning Calorimetry (DSC), Viscoamylography (RVA), X-ray Diffractometric Analysis (XRD), Scanning Electron Microscopy (SEM) and Colorimetry by Spectrophotometry.

1.9.1 Thermal Analysis

By definition, thermal analysis is a term that comprises the group of techniques that monitor the physical or chemical property of a substance or its reaction products. The monitoring takes place according to the time or temperature, under a specific atmosphere and controlled programming of temperature (GIOLITO; IONASHIRO, 1988). In the last two decades, there has been a growing diffusion of thermal methods, which have an extensive use in scientific research, mainly in studies that study the phenomenon of heat treatment in foods, in particular, the study of starch behaviour (BENINCA et al., 2013; SCHNITZLER et al., 2004).

Among the techniques used are Thermogravimetry and Derivative Thermogravimetry (TG, DTG), Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC), etc. These techniques provide information on mass variation, thermal stability, free water and bound water, purity, melting point, boiling point, transition heats, specific heats, phase diagrams, reaction kinetics, glass transition, etc. (GIOLITO, IONASHIRO, 1988).

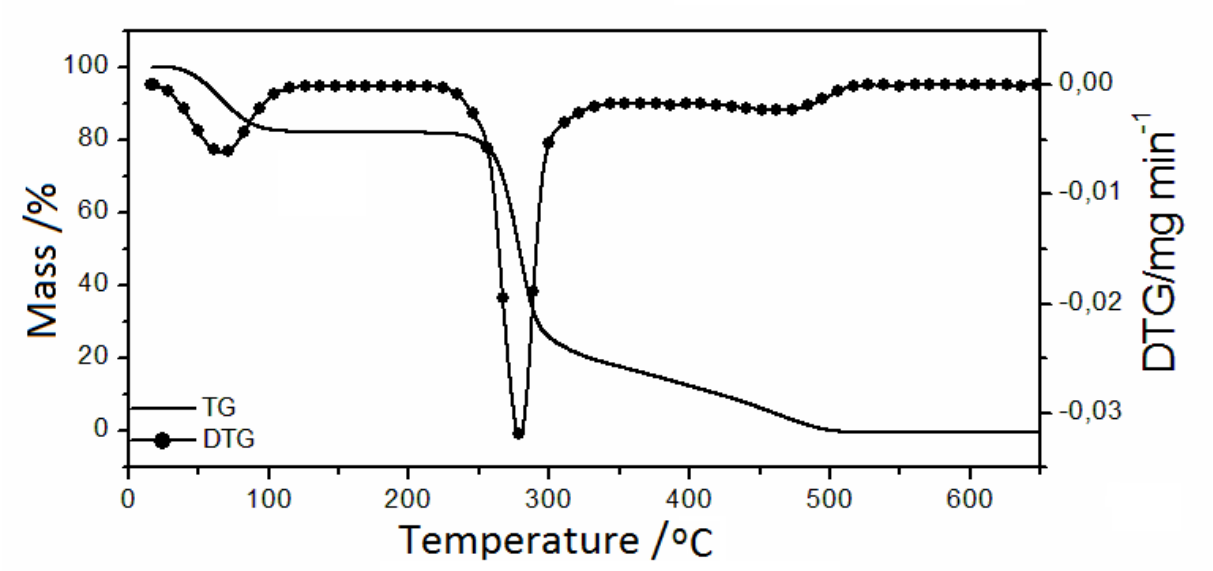
1.9.1.1 Thermogravimetry (TG) and Derivative Thermogravimetry (DTG)

Thermogravimetry is a technique in which a continuous recording of the mass loss of a substance is carried out in a controlled atmosphere, depending on the temperature (heating or cooling) or time, while the substance is subjected to controlled temperature programming (BENINCA et al., 2013).

The TG curve (Figure 8) graphically provides steps corresponding to mass losses as a function of time and temperature, and through the curve, it is possible to obtain information on stoichiometry, thermal stability, and composition, the stability of intermediate compounds and structure of the final product (GIOLITO, IONASHIRO, 1988). Derivative Thermogravimetry (DTG) is a mathematical resource obtained

through the first derivative of the TG curve as a function of time or temperature. The record is the derived thermogravimetric curve or DTG curve. In the DTG curve, the steps of the TG curve are matched by peaks that delimit areas proportional to the loss of mass in that thermal event (ANDRADE et al., 2013).

FIGURE 8. TG/DTG CURVE EXAMPLE OF CORN STARCH.



SOURCE: The author (2018).

1.9.1.2 Differential Scanning Calorimetry (DSC) and Differential Thermal Analysis (DTA)

Among the thermoanalytical methods, Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC) have proven most useful in providing basic information on starch (SHARMA; PRUNEAU, 2008).

Differential Scanning Calorimetry (DSC) is the technique in which the energy difference supplied to the sample and a reference material is recorded, generating a curve that shows exothermic or endothermic peaks (Figure 9) as a function of temperature while the substance and material are subjected to a controlled temperature programming (GIOLITO; IONASHIRO, 1988). Differential Thermal Analysis (DTA), on the contrary, measures the difference in the temperature between the sample and the reference. DSC has supplanted DTA as the method of choice for starch characterization (SHARMA; PRUNEAU, 2008).

Depending on the instrument used, and the purpose of the study DSC measurements can be performed isothermally or by heating and cooling. The

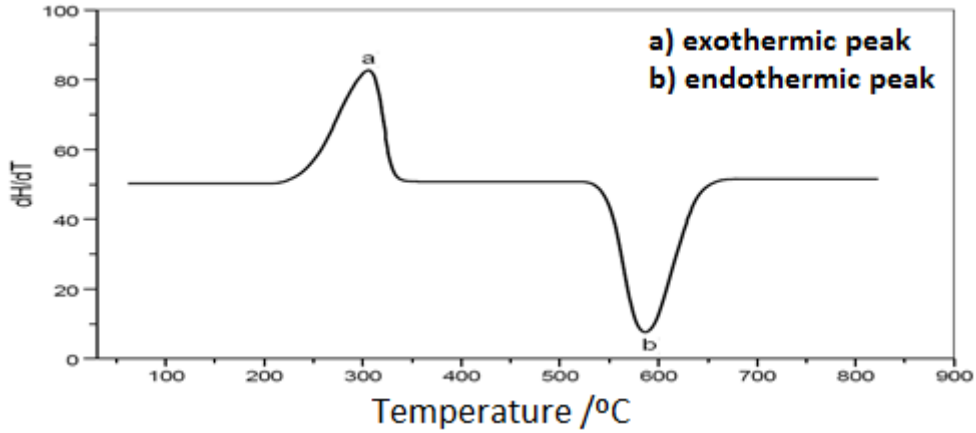
temperature to be used can be programmed according to the interest of the study (GIOLITO; IONASHIRO, 1988).

Differential Scanning Calorimetry (DSC) allows measuring the temperature and heat rate associated with transitions in materials as a function of time and temperature in a controlled atmosphere (SILVA, 2011). The main parameters determined are: the glass transition temperature Tg characterized by a change of the baseline in the DSC curve; specific heat (change of baseline); melting temperature characterized by an endothermic peak in the DSC curve; crystallization temperature (exothermic peak); oxidation (exothermic peak); degradation (peak endo or exothermic) (SILVA, 2011).

The glass transition is a phase transition that occurs in amorphous materials when subjected to a certain temperature (PEREZ, 1994). When subjected to temperatures below Tg, the materials are in the vitreous state and are characterized by low molecular mobility, which gives them some rigidity; above the Tg, they are in the rubbery state, with greater molecular mobility and, consequently, greater flexibility (PEREZ, 1994). According to Forssel et al., (1997), the glass transition temperature is the most important parameter in the definition of the mechanical properties of amorphous polymers, due to their importance in the recrystallization and storage processes of these materials.

The mechanical and barrier properties of the polymers are related to Tg, than the knowledge of the glass transition temperature of the edible films helps in choosing the best storage conditions, and the permeation to gases and water vapor through the films occurring above the Tg temperature, where the polymer chains are in greater motion (MALI; GROSSMANN; YAMASHITA, 2010; ROGERS, 1985). By knowing the glass transition temperature of the biofilm, it can be predicted its behaviour with the temperature change for specific applications (SKOOG; HOLLER; NIEMAN, 2002).

FIGURE 9. DSC CURVE PROFILE: A) EXOTHERMIC PEAK; B) ENDOTHERMIC PEAK.



SOURCE: GIOLITO; IONASHIRO (1988).

1.9.2 Starch Paste Properties (RVA)

According to Mestres and Rouau (1997), the heating of the starch in excess of water causes the loss of the crystalline structure, produces swelling of the granules and partial solubilization of the polymers, resulting in the appearance of the viscoelastic properties of the starch. Starch applications in food processing it is common for it to be suspended in water and subjected to heating. Depending on the severity of the heat treatment conditions (time, temperature, pressure and shear), moisture content and the presence of other constituents, the separation phase of amylose and amylopectin can also begin during processing, resulting in a heterogeneous compound (DAIUTO; CEREDA, 2006).

The rheological processes of the starch pastes can be evaluated using equipment known as viscoamylographers such as Brabender viscoamylographers and Rapid Viscosity Analyzer (RVA). The determined parameters are initial paste formation temperature, maximum viscosity or peak viscosity, final viscosity and tendency to retrograde.

The paste properties profile of the starches analyzed in the rapid visco-analyzer RVA, compared to the results of the enthalpies of gelatinization, can provide parameters related to the proportion and type of crystallinity.

1.9.3 X-ray Diffractometry (XRD)

For the characterization of materials there are several techniques, however, for the determination of crystalline phases the X-ray diffraction technique is the most indicated. This is due to the fact that in the crystals, the atoms are arranged in crystalline planes separated from each other by distances of the same order of magnitude of the X-ray wavelengths. X-rays are diffracted by a crystal because the electrons of their atoms absorb the radiation and then serve as secondary sources that re-emit radiation in all directions (ALBERS et al., 2002; LEIVAS et al., 2012). This technique is the best method to estimate crystallinity (LOUOUP et al., 1992).

Gallant et al. (1992) have described that the starch can be differentiated by the crystalline form and structure of its granules and visualized by X-ray diffraction patterns, being divided into three types or forms: the A-form of the starch, found in most foods, where, biochemically, there is a greater distance between the molecules of the starch granule that makes it more opaque and digestible; the form B, found in bananas, high amylose corn and raw potatoes, where, biochemically, the distance between the granule starch molecules is much smaller, and the granule becomes more crystalline, birefringent, compact and resistant to enzymatic hydrolysis; Form C has intermediate characteristics between forms A and B. There is still a pattern of X-ray diffraction of type V, which occurs when amylose molecules associate with lipids; this form of starch is partially resistant to enzymatic digestion (SHI; JEFFCOAT, 2001). The forms of the starch granules present in each food are a genetic characteristic of each plant cultivar.

Mondragon et al. (2004) observed the formation of the "V" type diffraction pattern (occurs when amylose molecules associate with lipids, this form of starch is partially resistant to enzymatic digestion), treated gelatinized maize starch with sodium hydroxide. However, Karim et al. (2008) treating sago starch with sodium hydroxide at concentrations of 0.1 and 0.5% did not observe the formation of the "V" type pattern and the standard remained as "C," characteristic for sago starch. The authors justify this difference by the low lipid content (<0.1%) of the sago starch, in addition to the alkaline treatment at room temperature.

1.9.4 Scanning Electron Microscope (SEM)

The starch granules have been subjected to structural investigations since the appearance of the microscope. The choice of technique and microscope for a high-resolution visualization of the structure of the granules depends on the type of information required, e.g., surface, or internal structure. Information on the surface of the starch granules can be achieved with both scanning electron microscopy (SEM) and atomic force microscopy (AFM). Information on the internal structure requires the use of transmission electron microscopy (TEM) (GALLANT et al., 1997).

Scanning electron microscopy is an important tool that among the various microscopic techniques has been employed in the study and characterization of starch granules. It has been widely used in research in the starch area, mainly in studies related to the shape and size of granules, chemical or physical changes of products.

1.9.5 Colorimetric Analysis

In all areas of scientific measurement has a relevant role since its reliability depends on manufacturing processes. In this way, the processes of measurement and standardization of colour become no less important. In industries, the standardization of colour confers product standardization, characteristic that, the customer infers quality. Colour analysis (colorimetry) can be performed either visually or instrumentally. In visual analysis (visual colorimetry) the colour of the sample is compared visually with the colour of a standard stored in a bottle of the same specification. This can be done under natural or artificial "white" light conditions or even in special chambers with various light sources (e.g., various wavelengths) (LEÔN et al., 2006; WILLIAMS; FEGOL, 1969; YAM PAPADAKIS, 2004).

Instrumental analysis replaces the human eye as a detector and can be done using photoelectric colorimetry or spectrophotometric colorimetry. Photoelectric colorimetry is the method that uses a photoelectric cell as a detector. It is usually employed with light contained within a relatively narrow range of wavelength obtained by the passage of white light through filters. The apparatus used in this method are known as colorimeters or filter photometers. Spectrophotometric colorimetry is the method that uses a source of radiation at various wavelengths in the spectral region

of the visible. The apparatus used in this method is known as a spectrophotometer (BRASIL, 2008; LEE et al., 2013, LEÓN et al., 2006).

CHAPTER 2

BRAZILIAN DIOSCOREACEAS STARCHES: THERMAL, STRUCTURAL AND RHEOLOGICAL PROPERTIES COMPARED TO COMMERCIAL STARCHES

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BRAZILIAN *DIOSCOREACEAS* STARCHES: THERMAL, STRUCTURAL AND RHEOLOGICAL PROPERTIES COMPARED TO COMMERCIAL STARCHES

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Abstract

Determination of the characteristics of native starches is crucial in order to select their best application in various industrial fields. Thus, two different types of non-traditional native starches from the *Dioscoreaceas* species (*Dioscorea sp*. and *Dioscorea piperifolia* Humb. var. Wild) were studied regarding their thermal, structural and rheological properties. The results were contrasted with traditional commercial starch sources (potato, cassava and corn). From the thermogravimetric results (TG/DTG) *D. piperifolia* starch obtained the highest thermal stability of the samples, except for potato starch. Furthermore, using differential scanning calorimetry (DSC) and viscoamylograph profiles (RVA) it was found that, the *Dioscoreaceas* starches presented a higher onset (T_o) temperature and susceptibility to retrogradation. They also showed lower values in relation to relative crystallinity, which was calculated from their X-ray patterns (XRD) and tendency to white (L^*) colour. The shapes of the *Discoreaceas* starch granules were determined using electron microscopy (SEM); it was found that as the potato starch the *Dioscoreaceas* starches showed a wide range of particle size.

Keywords: Yam native starch; unusual starch source; Rheological behavior; Thermogravimetric profile.

Introduction

In general, the food industry is the largest consumer of starch. However, starch is also used to process various products in the paper, chemicals, pharmaceutical and textile industries (HORNUNG et al., 2015; OLIVEIRA et al., 2014). Due to restrictions that have been mainly imposed by the food industry, and also because of the concerns about environmental issues, industries are increasingly interested in novel and underutilized sources of starch (HUANG et al., 2015).

Starch, is the most abundant carbohydrate reserve in plants and is also the main source of calories in the human diet. It is a non-toxic and renewable material. Products derived from starch have established applications in many fields. Starch granules are composed of two glucose polymers: amylose and amylopectin, which represent approximately 98 – 99 % of the dry total mass. Amylose is predominantly a linear polymer of *a* 1-4 linked glucose, whereas amylopectin is a highly-branched polysaccharide consisting of *a* 1-4 linked glucose with *a* 1-6 linkages at the branch points (HORNUNG et al., 2015).

The starch derived from cereal crops is the main form that is extracted and commercialised nowadays. Even though, plants widely used by humans (root tubers, rhizomes, bulbs) are rich in starch they are still underused. Brazil and others tropical countries have a large variety of tubers cultivars due to their mild climate (LEONEL; CEREDA, 2002). Tubers from the *Dioscoreaceae* family are estimated to number approximately 600 species (NASCIMENTO et al., 2015). *Dioscorea* tuber crops are cultivated in many parts of Africa and South East Asia. In Brazil the cultivation is mainly performed by traditional farmers for their own consumption and commercialized in the northeast of the country (NASCIMENTO et al., 2015; OKUNLOLA; ODEKU, 2011). In these tubers the starch is the most abundant constituent, making up to 20-60 % of total content; it serves as a sustainable source of starch for food and other industrial uses (JIANG et al., 2012; JIANG et al., 2013; FALADE; AYETIGBO, 2015).

Thermal analysis can be used to show the behaviour of starch granules when they are heated. For example, the differential scanning calorimetry (DSC) technique can be used to investigate the phase transitions of starch/water systems (gelatinisation process) and to estimate transition enthalpies (BICUDO et al., 2009). Furthermore, the structural configuration, paste and colour properties of starch play an important role in the development of food processing and new products (SANGSEETHONG; TERMVEJSAYANON; SRIROTH, 2010).

In this context, there is a need to investigate non-traditional starch sources which may be suitable for industrial use as additives to fulfill other specific purposes. Thus, the objective of this research was to study the thermal, rheological and structural properties of starch from *Dioscoreaceae* family tubers (*Dioscorea sp.* and *Dioscorea piperifolia* Humb. var. Wild) and compare them with commercialised starches.

Materials and Methods

The starches (500 g), from cassava (*Manihot esculenta*), corn (*Zea mays*) and potato (*Solanum tuberosum*) were purchased commercially in the city of Curitiba, Paraná, Brazil. The tubers of white yam (*Dioscorea sp.*) and aerial yam (*Dioscorea piperifolia* Humb. var. Wild.), which are presented in Fig. 1, were harvested from a crop area located in Aripuanã, Mato Grosso, Brazil. The tuber starches were obtained using the method describe in a previous study (LACERDA et al., 2014). The codification of the samples was as follows: samples of the native starches [white yam *D. sp.* – (a); aerial yam *D. piperifolia* – (b)]; commercial starches [potato – (c); cassava – (d) and corn – (e)].

FIGURE 1. PHOTOGRAPHY OF: A- *DIOSCOREA SP.* TUBER; B- *DIOSCOREA PIPERIFOLIA* HUMB. VAR. WILD. TUBER.

Source: The author (2018).

To obtain the thermal profile of the samples, in terms of mass loss under a controlled programmed temperature, the analysis was performed using TGA-50 thermal analysis system equipment (Shimadzu, Japan). All the mass loss percentages and the derivative thermogravimetric results were determined using TA-

60 WS data analysis software (HORNUNG et al., 2015; OLIVEIRA et al., 2014; CORDOBA et al., 2013).

The differential scanning calorimetry (DSC) analysis was performed using a thermal analysis system (TA-Instruments, DSC-Q200 model, USA). The system was previously calibrated with 99.99 % purity Indium, with a melting point of T_p. = 156.6 ºC, ΔH = 28.56 J g^{-1}. The thermal profiles were recorded under an air flow of 50 mL min^{-1} with a heating rate of 10 ºC min^{-1} and the samples, which weighed about 2.5 mg, were placed in sealed aluminum crucibles. A water:starch w/w (4:1) mixture was prepared and maintained for 60 minutes in order to equilibrate the moisture content (HORNUNG et al., 2015; OLIVEIRA et al., 2014; CORDOBA et al., 2013).

In order to study the pasting properties of the samples, the viscoamylograph profiles were obtained using a rapid viscometer (Newport Sci., RVA-4 model, Australia). A suspension of 2.24 g of starch in 25.76 g of distilled water underwent a controlled heating and cooling cycle following the methodology described in the literature (COLMAN; DEMIATE; SCHNITZLER, 2014; ANDRADE et al., 2014).

The morphology of the starch granules and their surface area were examined using a scanning electron microscope (Tescan, VEGA 3, Kohoutovice, Czech Republic) under an acceleration voltage of 15 kV and magnification of 1000x after the samples had been coated with plasma of gold and palladium (HORNUNG et al., 2015; OLIVEIRA et al., 2014; GRANZA et al., 2014). The average size of the starch granules was calculated using ImageJ free software (version 1.49v).

The X-ray diffraction powder patterns (XRD) of the samples were investigated using an X-ray diffractometer (Rigaku, Ultima 4 model, Japan) and the degree of relative crystallinity was quantitatively estimated using Eq. 1. The method followed was as described in the literature (COLMAN; DEMIATE; SCHNITZLER, 2014; BENINCA et al., 2013; ZHANG et al., 2009; ALBERTON et al., 2014).

$$Xc = \frac{Ap}{(Ap + Ab)} \text{ X } 100 \qquad (1)$$

where *Xc*, relative crystallinity, *Ap*, peak area, *Ab* base area.

A MiniScan EZ 4500L spectrophotometer (Hunter Inc., USA) was used to determine the colour parameters of the starch samples (*L**, brightness, which ranges from 0 (black) to 100 (white); *a**, positive values (red) and negative (green) and *b**,

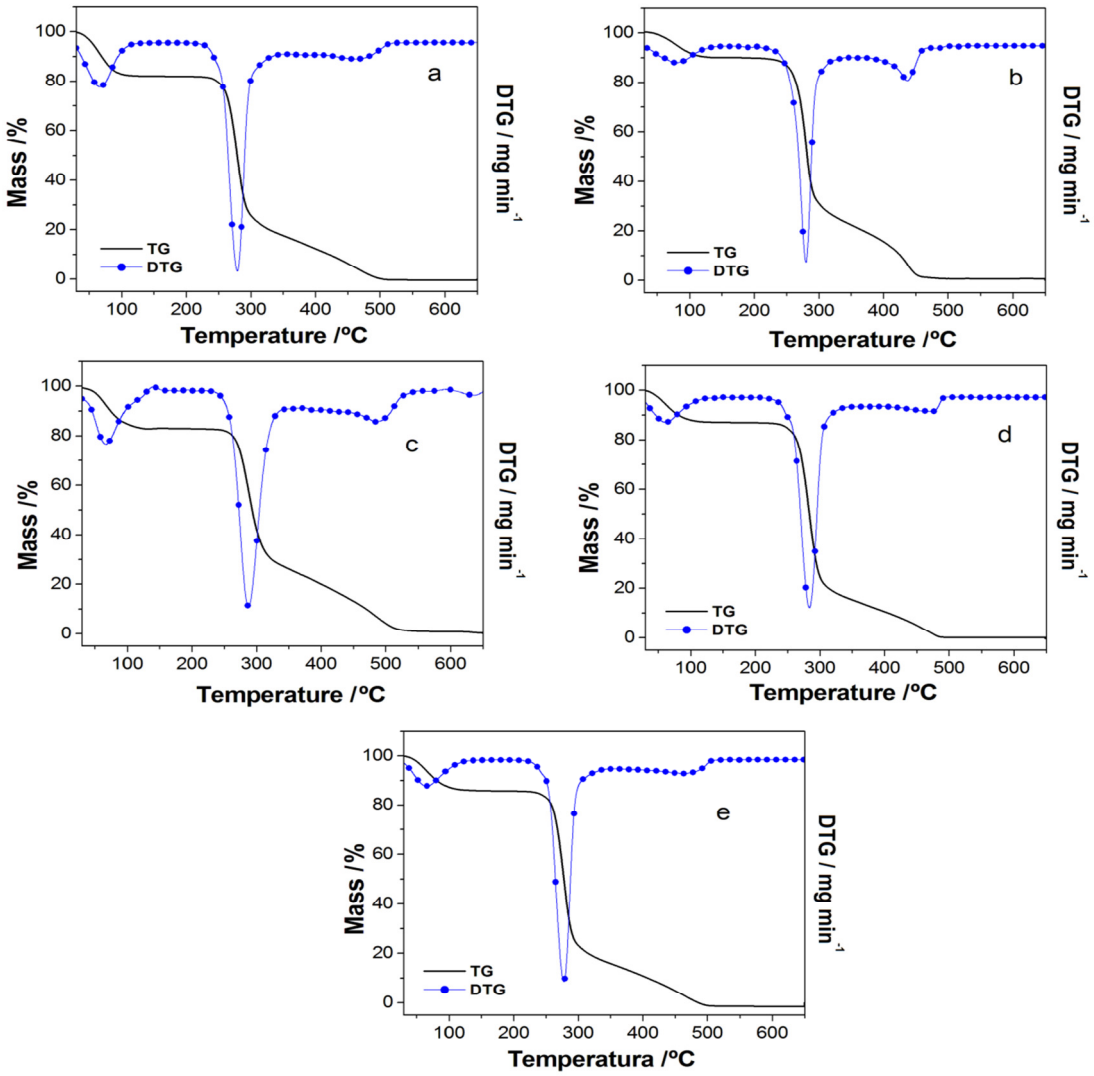
positive (yellow) to negative (blue) (HORNUNG et al., 2015; OLIVEIRA et al., 2014; COLMAN; DEMIATE; SCHNITZLER, 2014; HORNUNG et al., 2015).

All the results were analysed for variance (ANOVA). Duncan's test was used to compare the means of the samples at 95 % confidence level ($p<0.05$) utilising STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

The thermal decomposition of the starch samples occurred in three main steps, as can be observed in the curves shown in Fig. 2. The first step of mass loss, which as associated with the first peak followed by a stability step, was attributed to dehydration due to the water loss of the starch granules. The decomposition and oxidation of the organic matter and consecutive reactions were associated with the second and third steps, respectively (HORNUNG et al., 2015; CORDOBA et al., 2013; HORNUNG et al., 2015; ADAMOVICZ et al., 2015). Similar results were observed in previous studies (HORNUNG et al., 2015a; GRANZA et al., 2014; HORNUNG et al., 2015b; LACERDA et al., 2008; JAYAKODY et al., 2007; COOKE; GIDLEY, 1992; N'DA KOUAME et al., 2011).

FIGURE 2. TG AND DTG CURVES: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.



SOURCE: The author (2018).

Differences were observed regarding the derivative thermogravimetric profiles (DTG curves) of the studied samples. In relation to the first step (dehydration) it could be seen that the samples of white yam starch (a) and potato starch (c) presented a well-defined peak compared to the other samples; this can be attributed to the higher values for moisture content (a – 17.95 %; c – 15.89 %), Table 1. The moisture content of the samples b, d and e, were from 9 to 12%. Low moisture content can increase the stability of starches and it also facilitates transport and storage (COSTA et al., 2013). Furthermore, the third peak (oxidation) in the DTG curve (Fig. 2) of sample b (aerial yam) was different from the others. This could have been related to the slightly high depolymerisation (which occurs above 300 ºC under an oxidative atmosphere) of the starch molecules (ADAMOVICZ et al., 2015; LACERDA et al.,

2008; AGGARWAL; DOLLIMORE, 1998) compared with the other samples. Table 1 shows, the third event for sample b, which presented the highest mass loss (21.47 %) of all samples that were analyzed.

TABLE 1. TG AND DTG RESULTS: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.

Samples	**Step**	**TG Results**	**DTG Results**	
		Δm/%	**ΔT/ºC**	***Tp*/°C**
a	1st	17.95	30-103	66.27
	stability	-	103-227	-
	2nd	65.42	227-336	279.05
	3rd	15.99	336-516	457.67
b	1st	9.85	30-108	79.13
	stability	-	108-235	-
	2nd	67.79	235-372	280.06
	3rd	21.47	372-458	453.07
c	1st	15.89	30-105	72.54
	stability	-	105-242	-
	2nd	63.41	242-415	288.32
	3rd	19.87	415-532	479.87
d	1st	12.87	30-102	65.36
	stability	-	102-216	-
	2nd	72.46	216-392	283.61
	3rd	14.39	392-498	481.55
e	1st	12.11	30-101	65.67
	stability	-	101-232	-
	2nd	72.37	232-360	280.60
	3rd	14.13	360-506	481.82

* Δm mass loss (%), ΔT temperature range, *Tp* peak temperature

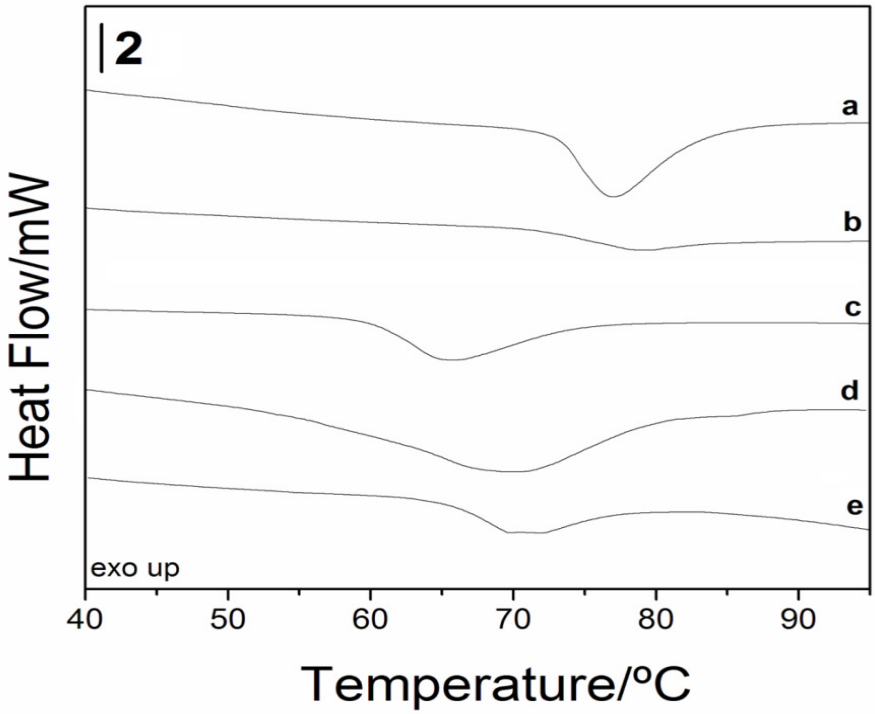
SOURCE: The author (2018).

The aerial yam starch (sample b), Table 1, presented a slightly higher range of stability step (108 – 235 ºC) when compared to the other samples, except for the potato starch. Thus, sample b could be considered a starch more stable to thermal degradation when compared to cassava and corn starches.

The results calculated from the TG/DTG curves are shown in Table 1. The residue corresponding to the mineral content of the samples was 0.64, 0.89, 0.83, 0.98 and 1.09 %, for samples a, b, c, d and e, respectively.

The DSC technique was used to study the gelatinisation process of the different starch samples. The profiles of the curves are depicted in Fig. 3, and Table 2 shows all the calculated results, which were carried out in triplicate.

FIGURE 3. DSC CURVES *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.



SOURCE: The author (2018).

TABLE 2. DSC, XRD AND SEM RESULTS: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.

Sample	DSC Results				XRD Results	SEM Results
	T_o / ºC	T_p / ºC	T_c / ºC	ΔH/J g^{-1}	*Degree of relative cristallinity (%)*	*Average size (µm)*
a	73.35 ± 0.01^{c}	76.92 ± 0.11^{d}	80.99 ± 0.07^{c}	15.56 ± 0.20^{c}	16.78 ± 0.02^{b}	29.57 ± 4.33^{c}
b	72.12 ± 0.01^{c}	78.88 ± 0.03^{d}	82.61 ± 0.07^{c}	10.05 ± 0.08^{b}	11.87 ± 0.05^{a}	31.14 ± 7.31^{d}
c	60.19 ± 0.02^{a}	65.49 ± 0.01^{a}	74.27 ± 0.03^{a}	12.07 ± 0.10^{c}	20.58 ± 0.02^{c}	35.55 ± 16.80^{e}
d	63.71 ± 0.05^{a}	70.51 ± 0.31^{b}	79.40 ± 1.03^{b}	9.63 ± 0.63^{a}	24.38 ± 0.03^{d}	13.83 ± 2.67^{b}
e	67.44 ± 0.03^{b}	73.23 ± 0.10^{c}	78.81 ± 0.46^{b}	8.49 ± 0.25^{a}	26.81 ± 0.03^{d}	12.95 ± 2.89^{a}

* T_o "onset" initial temperature, T_p peak temperature, T_c "endset" final temperature, ΔH_{gel} gelatinization enthalpy. ** Means followed by the same letter do not differ significantly by the Tukey test ($p < 0.05$).
SOURCE: The author (2018).

Fig. 3 shows the very different endothermic events (calorimetric curves) that occurred for the analysed samples. The initial temperatures, which were attributed to the change of the baseline, were quite similar for samples a and b (*Dioscoreaceas* starches) and also for samples c and d (potato and cassava).

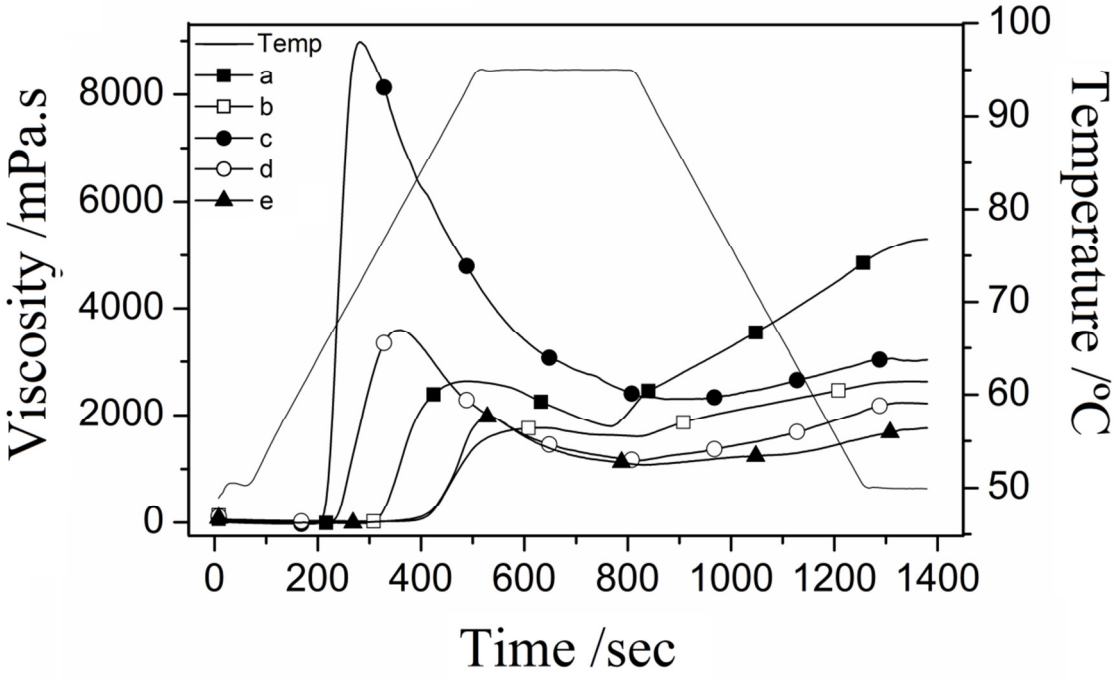
The gelatinisation process leads to irreversible changes in the structure of starch molecules, such as granules swelling and crystallite melting. As can be seen from the results in Table 2, the native starches from the *Dioscorea* species presented the highest values for the evolved temperatures of the gelatinisation process (T_o, T_p, T_c). These values were expected, due to the botanical sources of the yam starches which contain approximately 15 to 30% of amylose of its total starch (60 to 85 %) in dry basis (JAYAKODY et al., 2007). When starch contains a high amylose content it requires higher temperatures and a greater amount of energy for gelatinisation to occur. In solution, the linear structure of amylose assist in rapidly aligning with itself, resulting in more extensive hydrogen bonding and high gel strength. Thus, a high amount energy is required to break this bonds and gelatinise (KENSHUN;

ROSENTRATER, 2016). Similar results were obtained in previous studies of different species of yam starches (*Dioscoreaceas*) (N'DA KOUAME et al., 2011, PÉREZ ET AL., 2011; XIA ET AL., 2011; PÉREZ ET AL., 2013).

In the present study, the *Discorea* starch from the white yam cultivar (sample a) showed the highest Δ*H* value (15.56). A previous study obtained even higher Δ*H* values (17.32 to 18.98 J g^{-1}) for seven types of *Discorea* starches from Sri Lanka. Campbell, Berke and Glover (CAMPBELL et al., 1996) studied the influence of the variation of the size of starch granules on its functional properties. They found that starches with larger sized diameters particles can result in higher temperatures for DSC values. The *Dioscoreaceas* starches (samples a and b) had shown an average granule size of 29.57, 31.14 µm, respectively (Table 2), as well as higher Δ*H* values, as related above. Regarding the Δ*H* values obtained for sample b (*D. piperifolia*), this native starch consumed less energy to form the starch gel in comparison with the potato sample (35.55 µm).

One of the most important characteristics when recommending the technological application of amylaceous materials is viscosity. Viscosity curves can show the behaviour of the starch paste during heating and cooling (ZHOU et al., 2013). The pasting properties for the starch samples obtained by the RVA profiles are presented in Fig. 4 and the main results are shown in Table 3.

FIGURE 4. RVA PROFILES: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.



SOURCE: The author (2018).

TABLE 3. RVA RESULTS: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.

Sample	Pasting temperature /°C	Viscosity peak /mPa.s	Peak time /sec	Setback /mPa.s	Breakdown /mPa.s	Final viscosity /mPa.s
a	76.70 ± 0.28^{c}	2383.50 ± 0.71^{c}	420.05 ± 0.07^{c}	3943.00 ± 2.83^{a}	1007.50 ± 6.36^{c}	5324.50 ± 4.95^{a}
b	74.43 ± 0.04^{c}	1762.60 ± 0.85^{e}	628.28 ± 0.11^{a}	1024.00 ± 1.41^{b}	161.50 ± 2.12^{e}	2628.50 ± 2.12^{c}
c	64.87 ± 0.01^{a}	8982.30 ± 0.42^{a}	280.00± 1.41^{e}	723.73 ± 0.18^{d}	6675.05 ± 0.21^{a}	3031.12 ± 0.16^{b}
d	66.75 ± 0.07^{a}	3597.50 ± 2.12^{b}	348.55 ± 0.78^{d}	833.75 ± 2.47^{c}	2331.15 ± 1.63^{b}	2054.00 ± 5.66^{d}
e	70.58 ± 0.67^{b}	1972.40 ± 0.57^{d}	531.25 ± 1.06^{b}	681.10 ± 0.14^{e}	895.15 ± 0.21^{d}	1758.05 ± 0.07^{e}

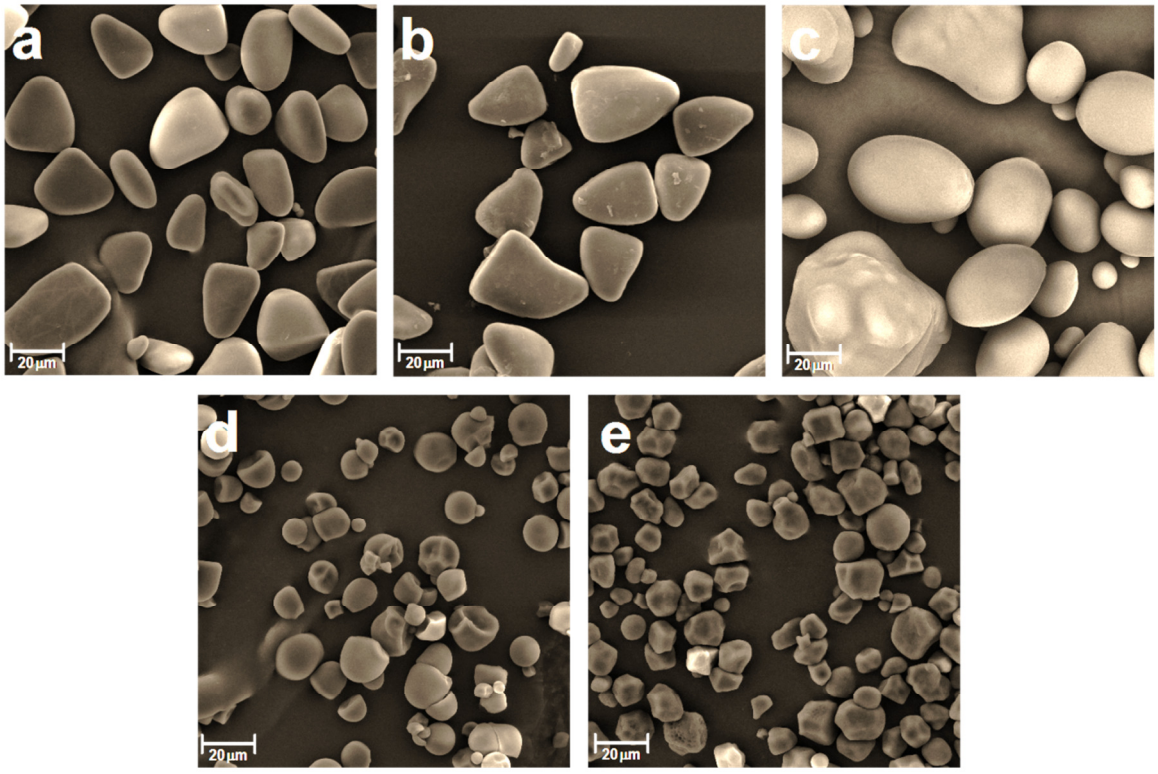
* mPa.s “millipascal-second”, sec “seconds”. ** Averages followed by the same letters in the same column do not differ statistically by Tukey test ($p < 0.05$).

SOURCE: The author (2018).

The pasting temperatures obtained by RVA corroborated the onset temperatures of gelatinisation that were registered by the DSC analysis. The higher values recorded by RVA are due to the fact that this technique is less sensitive in detecting when starch granules begin to disrupt their configuration (ZHOU et al., 2013). Compared to the commercial samples, the higher pasting temperatures presented by the yam starches (samples a and b) may have been related to a high resistance to the swelling and rupture of the starch granules. All the results for peak viscosity, peak time, setback, breakdown and final viscosity were significantly different between the samples. In spite of the fact that sample c (potato) showed a high value for peak viscosity (8982.30 ± 0.42 mPa.s) the highest value in terms of final viscosity was achieved for the white yam starch (sample a – 5324.50 ± 4.95 mPa.s). This result obtained for the white starch suggests that the native starch would be a better alternative than potato starch to be used in the food industry as a basis for soups, gravies, sauces, baby foods etc (WONGSAGONSUP et al., 2014). Highly viscous starch presents a heavy body, shorter texture and a lower level of breakdown in cooking, thereby functioning as a better food system stabiliser (WONGSAGONSUP et al., 2014). The setback parameter can provide an idea of the proportion of the partial association of the amylose and amylopectin macromolecules after the gelatinisation process (TOVAR et al., 2002). This association expels the

water molecules from the gel, forming hard pastes in a process known as retrogradation (TOVAR et al., 2002). Starches that have a higher tendency to retrogration (setback values), such as those presented by the *Dioscoreaceas* starches (samples a and b), are considered to be resistant starches (AALCAZAR-ALAY; MEIRELES, 2015). Starches that present such results can be applied as a basis for bioplastic material due to their ability to form cohesive films (SIMKOVÁ et al., 2013). Different results for setback are found in the literature regarding *Dioscorea* starch sources (JIANG et al., 2012; JAYAKODY et al., 2007; 38]. These differences can be attributed to the botanical origin, chemical composition, the method used and the conditions of analysis (ZHOU et al., 2013).

FIGURE 5. SEM IMAGES (WITH MAGNIFYING 1000X): *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.

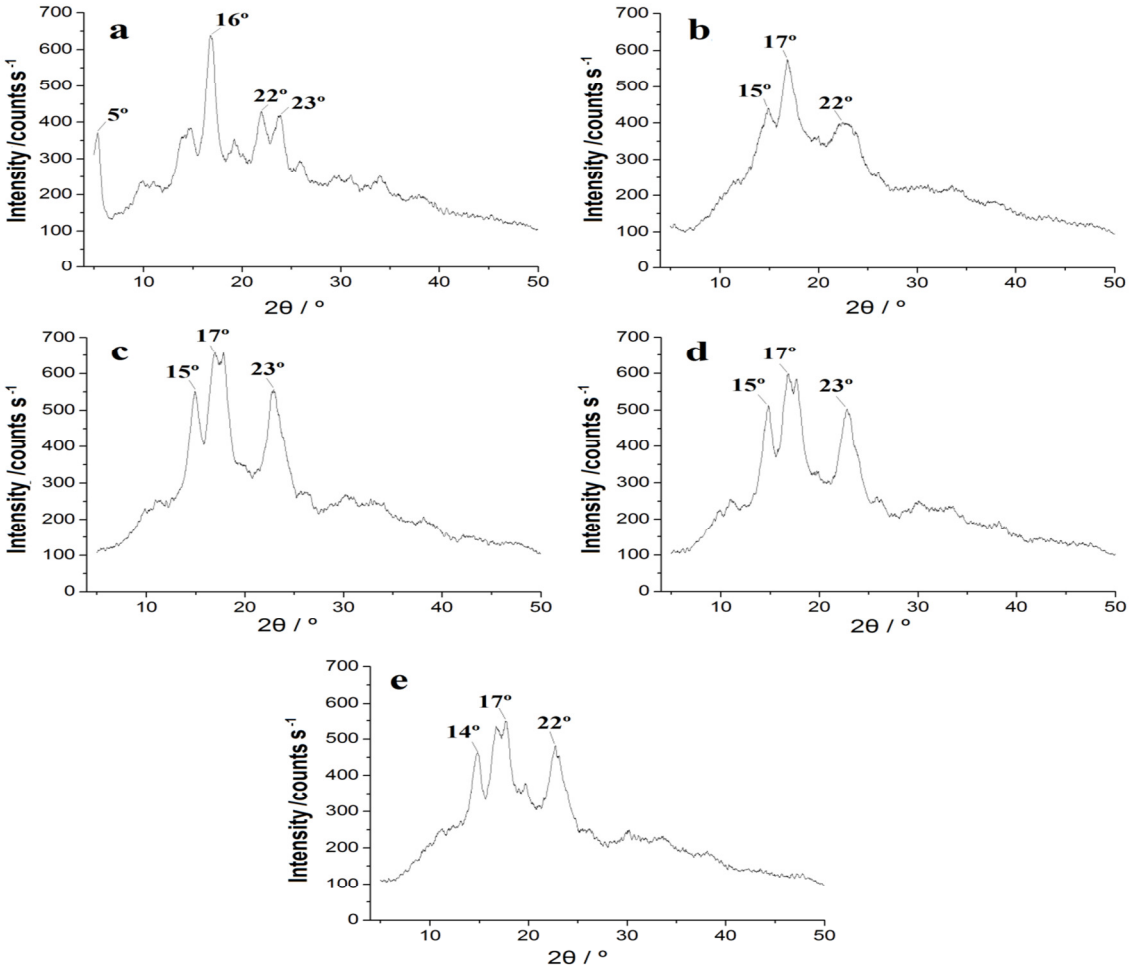


SOURCE: The author (2018).

Fig. 5 shows the morphological images of the studied samples acquired by SEM. The micrographs showed that the shapes and sizes of the starches granules for the analyzed samples differed significantly between themselves. The starch granules from samples a, b and c range from large to medium size, and samples a and b were predominantly triangular in shape. The results obtained for all the

samples were similar to those in previous studies (HUANG et al., 2015; JIANG et al., 2012; JIANG et al., 2013; FALADE; AYETIGBO, 2015; ADAMOVICZ et al., 2015; JAYAKODY et al., 2007; N'DA KOUAME et al., 2011). Based on the micrograph images of the starch granules the average size was calculated and the results are depicted in Table 2. In terms of these results, all samples analyzed presented significant differences. Sample a showed a range between 24 to 40 µm with an average size of 29.57 µm. Sample b ranged between 24 to 41 µm with an average size of 31.14. Determining the shape and size of starch particles is important in order to understand their technological behaviour (RILEY et al., 2008). For example, in the pharmaceutical industry the shape of starch granules influences their density during packing in a tableting machine or in filling gelatin capsule shells (RILEY et al., 2008). The pharmaceutical industry is interested in starch granules that show a wide range of particle size, such as the ones obtained for samples a, b and c (*Dioscoreaceas and potato* starches), which implies better technological performance (RILEY et al., 2008). The range of particles size for potato, cassava and corn starch were 11 to 49, 10 to 13 and 9 to 17 µm, respectively.

FIGURE 6. XRD DIFRATOGRAMS: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN.



SOURCE: The author (2018).

Observing the diffraction patterns (Fig. 6), regarding the main peaks the samples of the *Dioscoreaceas* species were different from the commercial starch samples. The diffraction patterns of samples a and b were very similar to the ones obtained in previous studies of *Dioscorea permsimilis* and *Dioscorea opposita* (JIANG et al., 2013; MALI; GROSSMANN; YAMACHITA, 2010). However, sample b showed a different pattern from a sample of the same species presented in the literature (JIANG et al., 2012). These differences can be attributed to the origin of the cultivars (climate, region, period of collection), the methodology used, and the data analysis. The patterns can be better analysed by the results presented in Table 2. Depending on the strongest diffraction peaks that are registered, starch can be categorised as follows: Type A (main peaks at 15, 17, 18 and 20º into 2*θ*); Type B (5, 6, 15, 17, 18 and 23º) or Type C (a mixture of types A and B = 5.5, 15, 17, 22 and 23º) the last one is normally attributed to rhizome starches (HORNUNG et al., 2015;

HUANG et al., 2015). Therefore, in this study the main peaks into 2*θ* presented for samples a (5, 16, 22 and 23º) and b (15, 17 and 22º) can be classified as type C starches as well as the ones obtained for the commercial samples, (Fig. 6).

The results for the degree of relative crystallinity showed that samples a and b were significantly different from the commercial starch samples. Furthermore, these *Dioscorea* starch samples showed the lowest values for relative crystallinity, which were 16.78 and 11.87 %, respectively. These results are different from the data obtained in the literature (JIANG et al., 2012; LIN et al., 2016) for *D. sp.* (35 %) and for *D. piperifolia* (32.06 %). These differences were mainly due to the composition of the starch, the moisture content and the botanical source (JIANG et al., 2013). These low values can be attributed to a high amylose content (JAYAKODY et al., 2007). Starches composed of large amounts of amylose molecules have great potential for the development of resistant edible films (OLI et al., 2016), and high-amylose starches are considered to be healthier for human consumption because of their resistance to digestion (AALCAZAR-ALAY; MEIRELES, 2015; CHUNG; CHANG; LIM, 2004). Considering the results for low relative crystallinity and high setback for samples a and b, the amount of amylose could contribute for the differences between those starches and the commercial samples. It is also relevant to mention that a low degree of relative crystallinity of a starch can increase the enthalpy (Δ*H*) of the gelatinisation process (RODRIGUES et al., 2012), as shown by sample a (*D. sp* – white yam), Table 2.

TABLE 4. COLORIMETRY RESULTS: *NATIVE* STARCHES A- *DIOSCOREA SP.* AND B- *DIOSCOREA PIPERIFOLIA*; COMMERCIAL STARHES C- POTATO, D- CASSAVA, E- CORN, RESPECTIVELY.

Sample	***Colour Parameters Results***		
	L*	***a****	***b****
a	85.88 ± 0.42^{b}	-0.20 ± 0.07^{a}	1.57 ± 0.30^{a}
b	72.36 ± 0.18^{a}	10.10 ± 0.03^{c}	35.65 ± 0.14^{d}
c	93.16 ± 0.59^{d}	-0.07 ± 0.03^{b}	3.85 ± 0.09^{b}
d	88.84 ± 0.33^{c}	0.27 ± 0.21^{d}	2.08 ± 0.31^{a}
e	93.41 ± 0.56^{d}	0.84 ± 0.04^{e}	5.51 ± 0.05^{c}

** Averages followed by the same letters in the same column do not differ statistically by Tukey test ($p < 0.05$).

SOURCE: The author (2018).

The results of the colour properties are presented in Table 4. Visually, all the starch samples were white, with the exception of the starch from sample b (*D. piperifolia*), which was yellow. The visual colour observation of sample b was confirmed by the results for the *b** parameter, which registered a positive value of + 35.65 (yellow) and the lowest value for brightness (*L**) of + 72.36. Sample a (*D. sp*) also presented a lower value for the *L** parameter when compared to the commercial starch samples although it showed the least tendency to yellow (*b** = 1.57) of all the samples. Similar results were found for these cultivars in previous studies (JIANG et al., 2012; JIANG et al., 2013; FALADE; AYETIGBO, 2015; JAYAKODY et al., 2007). Regarding sample (b) from the *Dioscorea piperifolia* starch, the presence of pigments like carotene in its tuber (FALADE; CHRISTOPHER, 2015) carried over to the starch, which may have been responsible for its colour properties. Starches that bring pigmentation from their source can be applied in product formulations without a negative impact on their colour characteristics because the crucial factor is the amount that is applied.

Conclusion

The TG/DTG curves showed that the yam starches obtained a higher stability step compared to the commercial starch samples of cassava and corn. The DSC and RVA results verified the initial temperature of the gelatinisation process. It was possible to observe that the *Dioscoreaceas* samples showed a strong tendency to retrogradation (RVA - setback) and that the starch sample from *D. sp* had the higher value for gelatinisation enthalpy. The XRD crystallinity patterns of type C starches were observed for the *Dioscoreaceas* samples, which also presented the lowest values for relative crystallinity. All the colour results for the samples were significantly different and the aerial yam was the most coloured sample, tending to yellow. From the characteristics displayed in this study, *Dioscoreaceas* starches could be useful as a raw material for different commercial applications.

Acknowledgements

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CHAPTER 3

ENHANCEMENT OF THE FUNCTIONAL PROPERTIES OF DIOSCOREACEAS NATIVE STARCHES: MIXTURE AS A GREEN MODIFICATION PROCESS

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Enhancement of the functional properties of *Dioscoreaceas* native starches: mixture as a green modification process

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Abstract

To study the effect of the mixing, three types of starch from *Dioscorea sp.*, *Dioscorea piperifolia* Humb. var. Wild. and *Dioscorea Trífida* tubers were mixed in different proportions and their thermal, structural and rheological properties were characterised. Using thermogravimetric (TG) and differential thermal analysis (DTA) it was found that all the mixed samples presented enhanced thermal stability. By differential scanning calorimetry (DSC) and viscoamylograph (RVA) techniques it was verified that the amylose content from the *D. sp.* starch influenced the gelatinisation and paste properties of the mixed samples. Furthermore, using scanning electron micrograph (SEM) analysis it was possible to suggest a correlation between the size of the starch granules and their relative degree of crystallinity, which was calculated by the X-ray diffraction (XRD) patterns. The colour analysis revealed that, irrespective of the proportion, the strong tendency to yellow of the native *Dioscorea piperifolia* predominated in the mixed samples.

Keywords: *Dioscorea* starch; starch modification; polysaccharides mixing; environmentally friendly process;

1 Introduction

Starch is a non-toxic and renewable material with established applications in many industrial fields (food, paper, chemicals, pharmaceutical and textiles) (MISMAN; AZURA; HAMID, 2015; HORNUNG et al., 2016, 2017; QIN et al., 2016). Starch presents glucose units in linear (amylose) and branched (amylopectin) molecules connected by α 1–4 linkages and α 1-6 linkages at the branched points (HORNUNG et al., 2016; KUUSISTO; MALONEY, 2016). Interest in alternative sources of starch from new and under-utilised starch cultivars is increasing (WANG et al., 2011; PINTO et al., 2015). Nevertheless, native starches have limitations, such as a high tendency to retrogradation, as well as low resistance to shear stress and thermal decomposition, for example (THYS et al., 2013).

To transform native starches into suitable ingredients, modifications processes are performed. Starch can be modified physically, genetically, enzymatically or chemically (ALBERTON et al., 2014; HORNUNG et al., 2015, 2016). With the growing pressure for natural products and environmentally friendly processes, mixtures of starches (from different sources or with other polysaccharides) represents an alternative that can enhance the properties of starches without using aggressive reagents or methods (ORTEGA-OJEDA; ELIASSON, 2001; HAGENIMANA; PU; DING, 2005; TUSKE; LÁSZLÓ; PINTYE-HÓDI, 2007; PARK et al., 2009; ZHANG et al., 2011; ELGADIR et al., 2012; ALBERTON et al., 2014; ADAMOVICZ et al., 2015; GUREVICH MESSINA; BONELLI; CUKIERMAN, 2015; HUSSAIN et al., 2016; JI et al., 2016; RUSS et al., 2016).

The *Dioscoreacea* family contains over 600 species of tubers (yams) which are widely cultivated in tropical and subtropical regions of the world. and are known for their high carbohydrate content and medicinal value (RILEY et al., 2008; ZHANG et al., 2016). These yams are very important for the food and pharmaceutical industries (CORNET et al., 2014). As a food crop, world production of yams reached 58.7 million tons and West Africa produces 92 % (Nigeria and Ghana – 66 %) of the world yam supply (AIGHEWI; MAROYA; ASIEDU, 2014). In Brazil, *Dioscorea* yams are mostly produced in the Amazon and Northeast regions (250,000 tons in 2008) (CASTRO et al., 2012; MENDES; SILVA; FAVERO, 2013; RAMOS et al., 2014). These tubers have great economic importance for the pharmaceutical industry as a raw material to produce diosgenin, which is a starting material for oral contraceptives,

sex hormones and other steroids (YANG; LU; HWANG, 2003; ZHANG et al., 2012; MOLLICA et al., 2013; WEI et al., 2016).

Although these tubers contain a starch content ranging from 70 % to 80 % of dry weight and they are a cheap potential source of starch for industry, these sources of starch are usually ignored (OKUNLOLA; ODEKU, 2011; ZHANG et al., 2012; HUANG et al., 2016). For example, 16,000 tons of starch, which is the residue from the production of 1000 tons of diosgenin, are discarded in China and drain into rivers causing serious pollution (YUAN et al., 2007; LIU et al., 2010; JIANG et al., 2012). Taking into consideration the amount of starch that is wasted in the production of diosgenin there is a need to study and enhance the functionality of *Dioscorea* native starches to provide potential industrial applications.

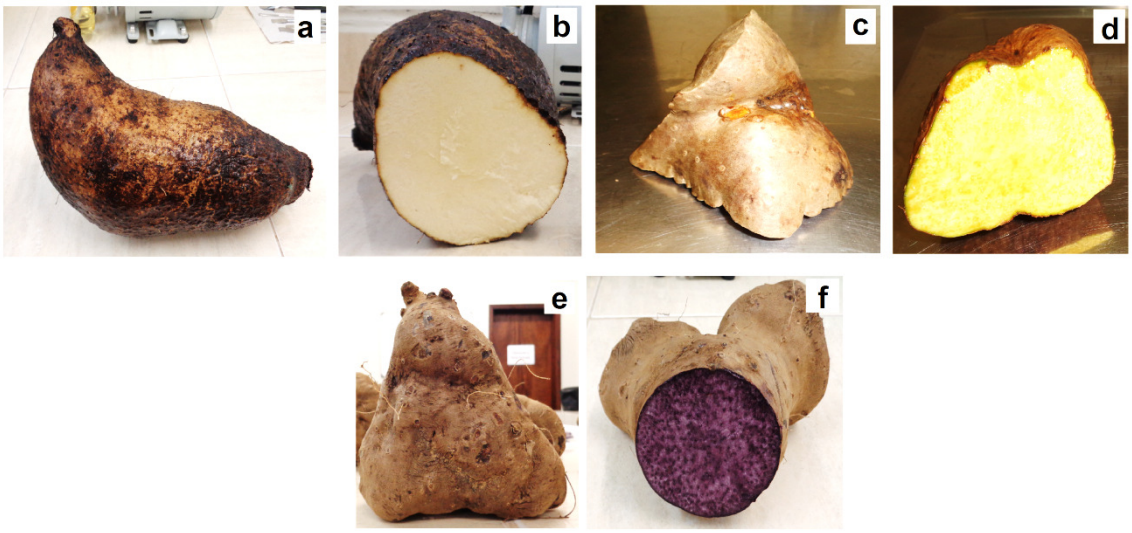
Compared to the two most commercialised tuber starches (potato and cassava) yam starches have smaller sized granules and are more suitable as fillers in biofilms and talcum powders, as well as providing important properties as thickening and gelling agents in foods (JAYAKODY et al., 2007b; ZHANG et al., 2012). *Dioscorea* starch granules consist of 10 – 30 % of amylose and 70 – 90 % of amylopectin, as well as other minor constituents (protein, lipids, phosphorous, saponins, phenolic compounds, etc.) (POLYCARP et al., 2012).

The purpose of the present study was to modify by mixing three different species of *Dioscoreaceas* starches from *Dioscorea sp.*, *Dioscorea piperifolia* Humb. var. Wild. and *Dioscorea Trífida*, and to characterise the thermal, rheological and structural properties of the native (unmixed) and the mixed starch samples.

2 Materials and methods

2.1 Materials

FIGURE 1. TUBERCLES IMAGES: A AND B – *DIOSCOREA SP.*; C AND D – *DIOSCOREA PIPERIFOLIA* HUMB. VAR. WILD; E AND F – *DIOSCOREA TRÍFIDA*.



SOURCE: The author (2018).

Dioscoreaceas tubers, Fig. 1, from the species *D. sp.*, *D. piperifolia* Humb. var. Wild. and *D. Trífida*, were harvested from a crop area in Aripuanã, Mato Grosso, Brazil (10º10'00" S / 59º27'34" W). The starch of each tuber was extracted as follows: the tubercles were washed, peeled and turned into pieces. The pieces were blended for three minutes with distilled water, the content was sieved (200 mesh) to separate the starch, and it was kept in decantation for 24 hours. The supernatant of the decanted starch was discarded and the starch was vacuum filtered and washed with distilled water. The filters containing the starch were dried at 36 ºC for 24 hours, sieved (200 mesh) and kept in a desiccator until the moment of analysis.

2.2 Mixtures

The mixtures (90 g) were prepared as follows: each portion of the starches (Table 1) was put together in a beaker glass (400 mL), suspended in distilled water (200 mL), and magnetically stirred for 15 minutes to ensure homogeneity. After stirring, the starches were vacuum filtered and dried for 24 hours at 36 ºC. After drying, the starches were ground and sieved (200 mesh) and kept in a desiccator until analysis. The starch samples were coded as follows: M1, M2, M3 (*D. sp.*, *D.*

piperifolia and *D. Trífida*, respectively) native (unmixed) samples. The mixed starch samples were coded M4 to M10, Table 1.

TABLE 1. PERCENTAGES OF THE *DIOSCOREA* STARCHES USED IN THE MIXTURES.

Samples	Starch content (%)		
	D. sp.	*D. piperifolia*	*D. trífida*
M1	100	0	0
M2	0	100	0
M3	0	0	100
M4	50	50	0
M5	50	0	50
M6	0	50	50
M7	33	33	33
M8	70	15	15
M9	15	70	15
M10	15	15	70

D. – Dioscorea.

SOURCE: The author (2018).

2.3 Apparent amylose content

The apparent amylose content of the native *Dioscorea* starches were analysed using a iodine colorimetric method, as described by (MARTÍNEZ; CUEVAS, 1989).

2.4 Thermal properties

The thermal characteristics of the native and mixed samples were studied by using DTG-60H (Shimadzu, Japan) equipment that simultaneously provided thermogravimetric (TG) and differential thermal analysis (DTA). The gelatinisation properties were obtained using a differential scanning calorimeter (DSC-60A, Shimadzu, Japan). The TG-DTA and DSC analyses were carried out using the methodology and conditions described by (HORNUNG et al., 2015).

2.4.1 Pasting properties

The pasting properties of the starch samples were determined using a rapid visco analyser, RVA model 4 (Newport Scientific, Australia). The conditions and proceedings that were applied followed those outlined in our previous publication (HORNUNG et al., 2016).

2.5 Morphological analysis

2.5.1 X-ray diffraction (XRD)

The diffraction patterns of the native and the mixed samples were obtained using an X-ray diffractometer (Rigaku, Ultima 4 model, Japan) and the degree of relative crystallinity was quantitatively estimated as described in the literature (HORNUNG et al., 2016; QIN et al., 2016).

2.5.2 Morphological profile of granules

The morphological properties of the granules of the starch samples were observed using a scanning electron microscope, SEM (Tescan, VEGA 3, Kohoutovice, Czech Republic) under an acceleration voltage of 15 kV, a reading scale of 20 µm and magnification of 1000x after the samples had been coated with plasma of gold and palladium (BISINELLA et al., 2016; HORNUNG et al., 2016). The average size of the starch granules was calculated using ImageJ free software (version 1.49v).

2.6 Colour analysis

The colour analysis of the samples was performed using the CIE- L^* a^* b^* system. A MiniScan EZ 4500L spectrophotometer (Hunter Inc., USA) was used (DE MORAES CRIZEL et al., 2016).

2.7 Statistical processing

All the results were analysed for variance (ANOVA). Duncan's test was used to compare the means of the samples at 95% confidence level ($p<0.05$) utilising STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

3 Results and discussion

3.1 Apparent amylose content

The apparent amylose content (%) of the native *Dioscorea* starches was 28; 12; and 13.4 for the *D. sp.*; *D. piperifolia* and *D. trífida* samples, respectively. Proximate results were found for *D. sp* (26.7 %; 23.6 %) in a study by (PÉREZ et al., 2013), which was determined by iodine biding capacity and DSC. Different results were found by (PÉREZ et al., 2011) for *D. trifida* in their study of three genotypes of *D. trífida* from the Venezuelan Amazon. The aforementioned authors performed three different methodologies (colorimetric, DSC and amperometric), which presented results ranging from 1.4 to 12 %. No data was found in the literature regarding the amylose content of *D. piperifolia*. The differences between the results may have been due to the cultivars, genotype, area and climate of collection, as well as the methods and proceedings that were used (YUAN et al., 2007; PÉREZ et al., 2011).

The determination of amylose content is important because it has an impact on the properties of starch, such as crystallinity, gelatinisation temperatures, pasting behaviour, as well as the mechanical properties of starch films etc (STAWSKI, 2008; CHUNG et al., 2011).

3.2 Thermal properties

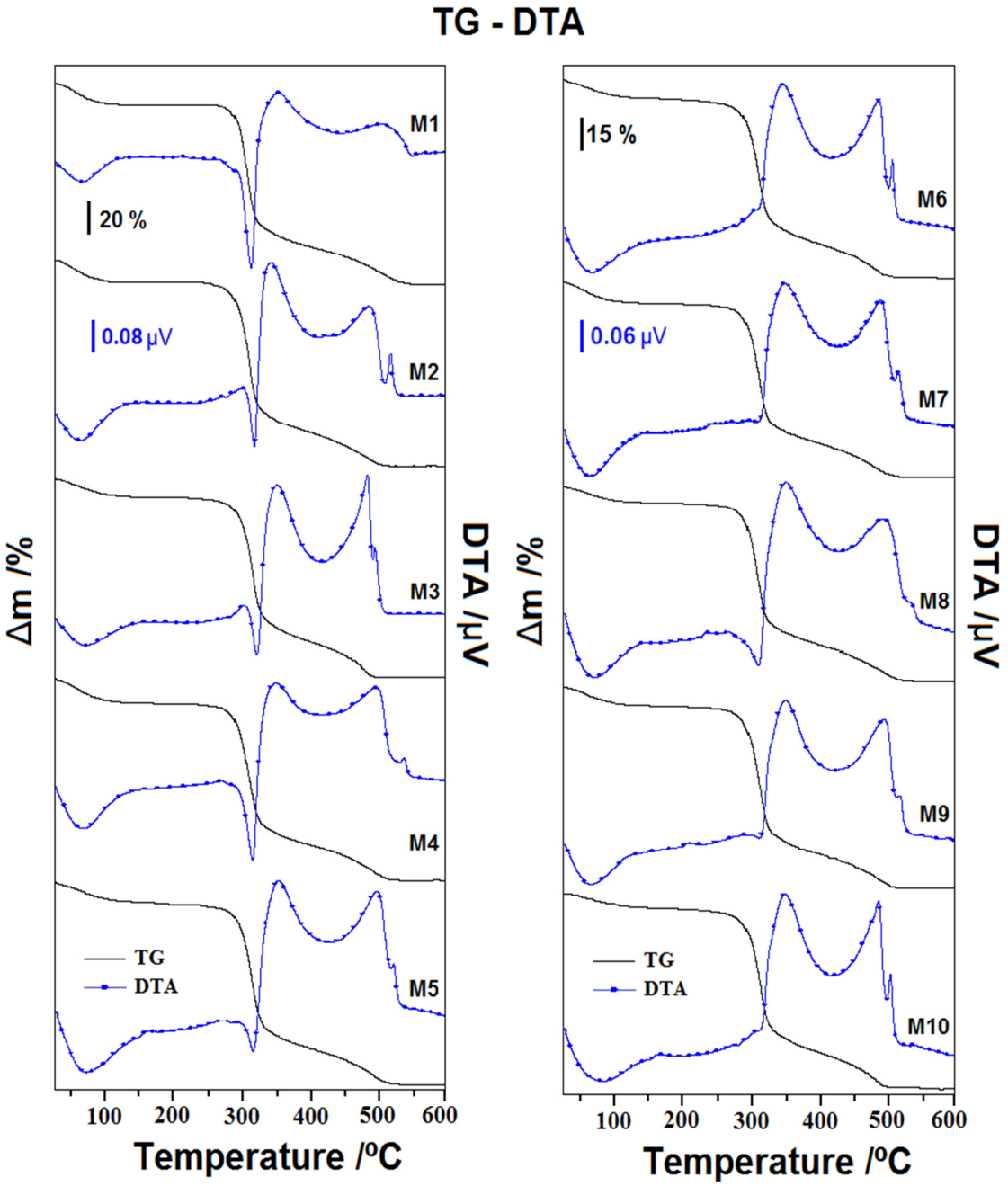
3.2.1 Thermogravimetry (TG) and differential thermal analysis (DTA)

The TG-DTA profiles of the native and mixed samples are presented in Fig. 2 and the results are shown in Table 2. The TG curves for the samples showed that thermal decomposition occurred in three main steps of mass loss. The first mass loss started at 30 ºC and occurred from 102 to 112 ºC. This first step was associated with dehydration and the loss of volatile compounds, which was followed by a stability step. Low moisture content was presented by the samples, which ranged from 5.6 to 11.4 %; this low content can extend the shelf life of starch and facilitate its storage and transport (COSTA et al., 2013). In the determination of moisture content, thermogravimetric analysis has advantages compared to the classical gravimetric

methods because it requires a smaller amount of samples, as well as being faster and more accurate (COSTA et al., 2013).

Once the evaporation of the water occurred, the second and third steps were attributed to the degradation and oxidation of the organic matter, which formed a final residue (ash ≅ 1.2 % for all samples with an initial mass of ≅ 5 mg).

FIGURE 2. TG AND DTA CURVES OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.



SOURCE: The author (2018).

The DTA curves showed that the second and third steps of mass loss were consecutive and began with an endothermic reaction followed by three exothermic reactions. The third exothermic peak is most probably connected with thermal decomposition of amylopectin since both samples which contained more amylose (M1, M8) exhibited only two exothermic peaks. Samples M6, M7 and M10 did not show any endothermic reaction. Similar thermogravimetric profiles were found in previous studies regarding potato starch and pectin mixture (ADAMOVICZ et al., 2015); the co-pyrolysis of peanut shells and cassava starch mixtures (GUREVICH MESSINA; BONELLI; CUKIERMAN, 2015); the influence of some sugars on the properties of *pinhão* starch (RIBEIRO et al., 2014); the properties of Brazilian *Dioscoreaceas* starches compared to commercial starches (HORNUNG et al., 2017); and native and oxidised starches from two varieties of Peruvian carrot (MATSUGUMA et al., 2009).

TABLE 2. TG AND DTA RESULTS OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.

Samples	TG Results		DTA Results		Samples	TG Results		DTA Results	
	Step	Δ*m* /%	Δ*T* /ºC	*Tp* /°C		Step	Δ*m* /%	Δ*T* /ºC	*Tp* /°C
M1	1st	8.5	30 – 112	70.0	**M6**	1st	8.3	30 – 104	72.0
	Stability	-	112 – 265	-		Stability	-	104 – 270	-
	2nd	76.00	265 – 439	315.0 355.0		2nd	77.3	270 – 412	348.2
	3rd	17.8	439 – 567	503.0		3rd	11.9	429 – 568	488.0 509.0
M2	1st	7.9	30 – 109	69.0	**M7**	1st	11.4	30 – 102	68.5
	Stability	-	109 – 255	-		Stability	-	102 – 269	-
	2nd	69.7	255 – 448	321.0 345.0		2nd	72.00	269 – 415	350.5
	3rd	21.2	448 – 539	520.0		3rd	15.5	415 – 581	490.1 516.4
M3	1st	8.8	30 – 110	73.3	**M8**	1st	6.7	30 – 105	74.9
	Stability	-	110 – 260	-		Stability	-	105 – 272	-
	2nd	74.4	260 – 416	322.0 351.0		2nd	78.6	272 – 422	312.5 352.6
	3rd	15.4	416 – 527	483.0 494.0		3rd	12.8	422 – 590	495.1 535.4
M4	1st	7.3	30 – 106	71.0	**M9**	1st	8.5	30 – 108	69.8
	Stability	-	106 – 268	-		Stability	-	108 – 272	-
	2nd	71.6	268 – 420	317.0 351.0		2nd	72.00	272 – 414	351.8
	3rd	19.7	420 – 593	497.0 538.0		3rd	18.00	414 - 579	496.1 518.8
M5	1st	9.1	30 – 103	73.0	**M10**	1st	5.6	30 – 105	86.3
	Stability	-	103 – 269	-		Stability	-	105 – 267	-
	2nd	79.9	269 – 428	315.0 353.0		2nd	78.5	267 – 415	350.8
	3rd	9.8	428 – 594	496.0 520.0		3rd	14.8	415 - 596	487.5 504.0

Δ*m* mass loss (%), Δ*T* temperature range, *Tp* peak temperature

SOURCE: The author (2018).

The thermal stability of a substance is attributed to its ability to maintain its properties as nearly unchanged as possible on heating and the TG - DTA is a useful technique for studying these attribute under a variety of conditions (WENDLANDT, 1986).

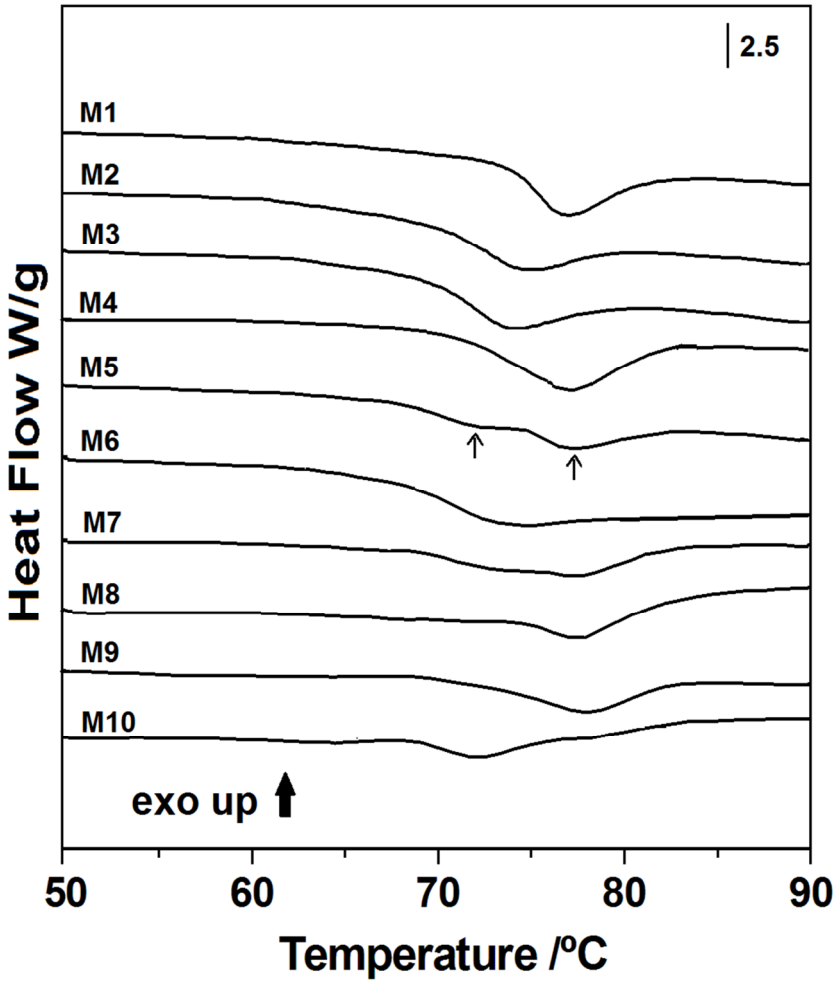
Table 2 shows the stability step values (DTA - ΔT temperature range) and compares the native samples (M1 *D sp.* starch: 112 - 265 ºC; M2 *D. piperifolia*: 109 - 255 ºC and M3 *D. trífida*: 110 - 260 ºC) and the mixed samples. A slightly higher range was recorded for samples M4 (106 – 268 ºC); M5 (103 – 269 ºC) and M7 (102 -269 ºC). The highest range was obtained for samples M6 (50/50 % starch content of *D. piperifolia* and *D. trífida*, respectively: 104 -270 ºC); M8 (70/15/15 % of *D sp.*; *D. piperifolia* and *D. trífida*: 105 -272 ºC) and for M9 (15/70/15 % of *D sp.*; *D. piperifolia* and *D. trífida*: 108 – 272 ºC). The increase in the final temperature of the range (stability step) was attributed to a resistance to thermal degradation. A study by (ZHANG et al., 2012) regarding the effects of ultrasound on the acetylation of *Dioscorea zingiberensis* C.H. Wright starch found lower values (average range of 105 – 245 ºC) for the samples that were analysed, except for sample “a” (125 – 276 ºC). A similar situation was found in the literature (VASHISHT; PANDEY; JAYARAM KUMAR, 2015); in this case the authors modified *Dioscorea* starches from Jharkhand by carboxymethylation using NaOH, isopropyl alcohol, monochloroacetate, methanol, acetic acid and acetone. The study of the thermal stability of starches is neccessary so that these polymers can be successfully used in various industrial applications (VASHISHT; PANDEY; JAYARAM KUMAR, 2015). Considering the TG-DTA results in the present study it could be said that the mixing that was performed altered the thermogravimetric profile of the native *Dioscoreaceas* starches without using any reagents that are hazardous to the environment.

3.2.2 Differential scanning calorimetry (DSC)

A very important property for the industrial application of starch is its gelatinisation process, i.e. the transitional phase of the starch granules from an ordered to a disordered configuration of molecules during heating with an excess of water (RIBEIRO et al., 2014). The DSC technique is a valuable tool to analyse the gelatinisation process because it reveals the transition temperatures and enthalpies,

which are related to the starch source, water content, ratio of amylose and measurement conditions (LIU et al., 2006).

FIGURE 3. DSC CURVES OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.



SOURCE: The author (2018).

The DSC curve profiles of the studied samples are presented in Fig. 3 and the calculated results are shown in Table 3. Fig. 3 shows that all the samples presented different curve profiles and that samples M5 (50/50 % of *D. sp.* and *D. trífida*, respectively) and M7 (33/33/33 % of each of the studied native samples) presented two endothermic events (the first one can be slightly perceived and is marked in Fig. 3). According to (ORTEGA-OJEDA; ELIASSON, 2001; HAGENIMANA; PU; DING, 2005), the endothermic events that were identified for samples M5 and M7 in the present study may have been due to the independent gelatinisation of each starch in the mixture. The calculated results shown in Table 3 are the sum of the two individual events. Previous literatures (HAGENIMANA; PU; DING, 2005; LIU et al., 2006) also

related this curve profile for the amylose and amylopectin ratio, granular architecture, extent of the branching points, units of chain length and polydispersity of amylopectin. Similar results were also reported by (JENKINS; DONALD, 1998; ORTEGA-OJEDA; ELIASSON, 2001; HAGENIMANA; PU; DING, 2005; LIU et al., 2006; ZAIDUL et al., 2008; ALBERTON et al., 2014; FALADE; CHRISTOPHER, 2015; GUREVICH MESSINA; BONELLI; CUKIERMAN, 2015). The other mixture samples (M4, M6, M8, M9 and M10) only presented an endothermic peak, and it could be said that the starch content, in each specific proportion, presented a homogeneous interaction between its constituents (ELGADIR et al., 2012).

TABLE 3. DSC, XRD AND SEM RESULTS OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.

Samples	DSC Results				XRD Results	SEM Results	
	T_o / ºC	T_p / ºC	T_e / ºC	ΔH_{gel} / J g^{-1}	*Degree of relative cristallinity (%)*	*Average size (μm)*	*Granules size range (μm)*
M1	74.00 ± 0.8^{c}	76.9 ± 0.1^{e}	81.3 ± 0.2^{b}	15.6 ± 0.6^{a}	15.5 ± 0.1^{b}	31.00 ± 7.0^{abc}	17 – 43
M2	70.2 ± 0.1^{e}	74.9 ± 0.1^{h}	79.2 ± 0.1^{c}	12.4 ± 0.8^{c}	24.6 ± 0.3^{f}	37.7 ± 12.1^{a}	17 – 57
M3	69.8 ± 0.2^{f}	73.7 ± 0.1^{i}	78.8 ± 0.3^{d}	12.6 ± 0.7	25.4 ± 0.1^{f}	35.7 ± 9.1^{a}	18 – 44
M4	71.3 ± 0.2^{d}	76.8 ± 0.1^{f}	81.9 ± 0.1^{a}	13.7 ± 0.5	19.6 ± 0.1^{a}	19.7 ± 9.4^{d}	8 – 38
M5	76.00 ± 0.4^{a}	77.7 ± 0.1^{b}	81.7 ± 0.1^{a}	8.6 ± 0.1^{e}	17.5 ± 0.4^{c}	21.6 ± 7.4^{cd}	6 – 28
M6	69.4 ± 0.1^{g}	75.1 ± 0.1^{g}	77.5 ± 0.2^{e}	6.00 ± 0.3	16.7 ± 0.2^{e}	32.8 ± 13.9^{ab}	15 – 44
M7	68.9 ± 0.1^{h}	77.4 ± 0.1^{c}	81.7 ± 0.2^{a}	9.6 ± 0.3	16.4 ± 0.2^{e}	27.2 ± 9.7^{abcd}	10 – 36
M8	75.1 ± 0.1^{b}	77.3 ± 0.1^{d}	81.3 ± 0.2^{b}	7.2 ± 0.^{f}	12.7 ± 0.2^{g}	30.3 ± 14.2^{abcd}	6 – 52
M9	73.9 ± 0.1^{c}	77.8 ± 0.2^{a}	81.8 ± 0.1^{a}	7.8 ± 0.1^{f}	15.3 ± 0.1^{f}	24.6 ± 5.5^{bcd}	14 – 31
M10	68.6 ± 0.1^{i}	72.00 ± 0.2^{j}	73.7 ± 0.1^{f}	3.8 ± 0.2^{h}	17.1 ± 0.1^{d}	24.8 ± 10.9^{bcd}	6 – 41

T_o "onset" initial temperature, T_p peak temperature, T_c "endset" final temperature, ΔH_{gel} gelatinization enthalpy. Means followed by the same letter do not differ significantly by the Duncan test ($p < 0.05$).
SOURCE: The author (2018).

The gelatinisation temperatures (onset *To*, peak *Tp*, endset *Te*) and the enthalpies (ΔH_{gel}) depicted in Table 3 show that there were significant differences between the native *Dioscoreaceas* starch samples and the mixtures. Among the native starches the onset temperature was highest for the native sample M1 (*D. sp.*), which also presented the highest content of amylose, and also for the mixtures containing *D. sp.* native starch, i.e. 50% (M4 71.3 ºC; M5 76.00 ºC) and 70% (M8 75.1 ºC). The amylose content plays an important role in the gelatinisation of starch. Literatures (FREDRIKSSON et al., 1998; MATSUGUMA et al., 2009) have pointed out that amylose and the amylose-lipid complex act as a restraint to swelling and resist the formation of the starch gel. Similar gelatinisation temperatures were found

in other studies of different species of *Dioscorea* starches (JAYAKODY et al., 2007b; YUAN et al., 2007; PÉREZ et al., 2011, 2013, JIANG et al., 2012, 2013).

The ΔH_{gel} refers to the measure of the overall crystallinity of amylopectin (TESTER et al., 1994) and it reflects the loss of the double-helix order (COOKE; GIDLEY, 1992) when starch is heated in excess water. A previous study (FREDRIKSSON et al., 1998) stated that the higher the amylose content in a starch, the higher will be the enthalpy required for gelatinisation to occur and the lower the crystallinity the starch will present. This can be seen from ΔH_{gel} obtained from DSC measurements and XRD relative crystallinity results (Table 3) for the native samples and the influence of its content on each mixture.

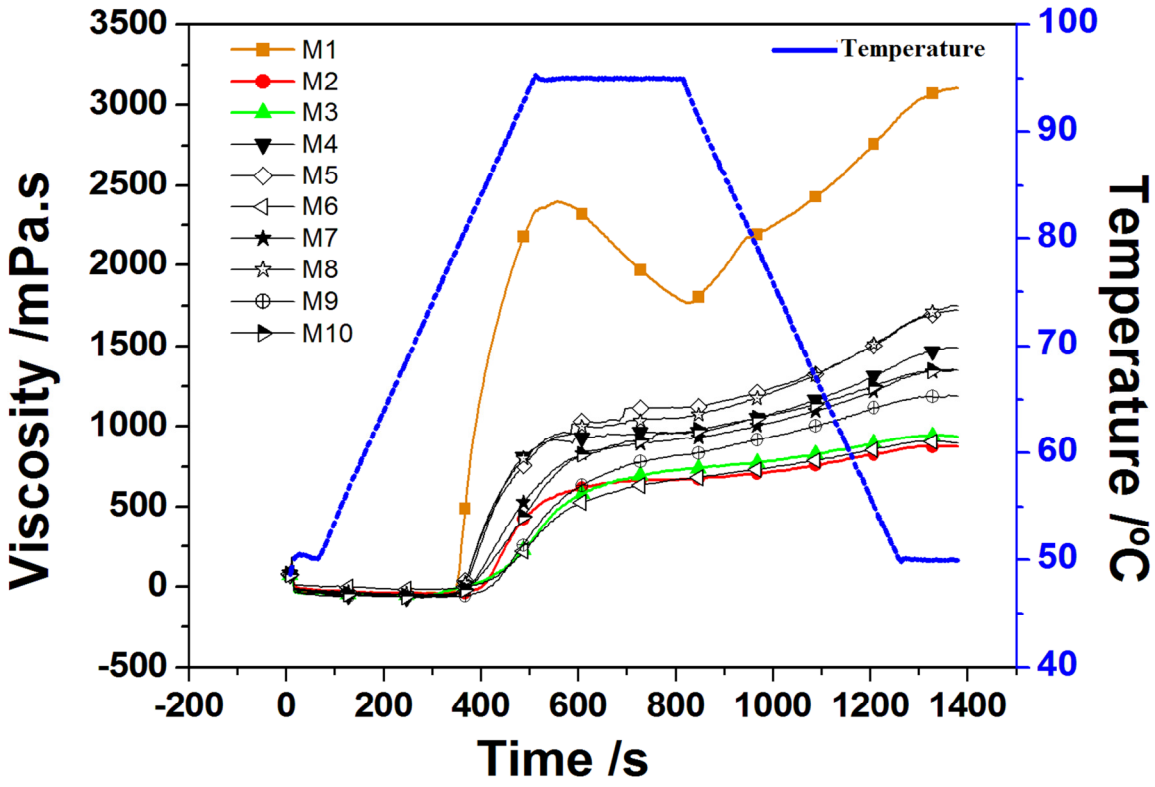
Observing the results (curve profiles, calculated results) obtained for the mixed samples and for the native samples it can be said that the proportions of mixtures altered the thermal behaviour of the native *Dioscoreaceas* starches. It is noteworthy that there was a large decrease in the amount of energy required for the gelatinisation process (ΔH_{gel}) for samples M5 to M9 (6.00 to 9.6) and that sample M10 (3.8) presented the highest decrease related to the native samples M1 (15.6), M2 (12.4) and M3 (12.6). Moreover, the proportions of starch mixture used in the M5, M6, M8 and M10 samples required less energy to form the gel than the different varieties of *Dioscorea sp.* starches that were annealed for 72 hours at 55 ºC by (JAYAKODY et al., 2007a), where only the java-ala starch type exhibited 7.8 (ΔH_{gel}) and the other varieties ranged from 18.1 to 19.5 (ΔH_{gel}).

3.3 Pasting properties

Starch has many important functional properties including pasting behaviour, which can be obtained by analysis performed on a rapid visco analyser (RVA), as well as several other techniques. The characteristics that can be studied regarding starch paste relate to viscosity changes based on rheological principles. Several parameters can be obtained from the RVA curve profiles that are intrinsically related to the starch constituents, principally the amylose/amylopectin ratio, phosphorous and lipid content, etc. (ZAIDUL et al., 2007; MATSUGUMA et al., 2009; HORNUNG et al., 2015, 2016, 2017).

The RVA curves of the starch samples are presented in Fig. 4 and the calculated results for the pasting properties are shown in Table 4.

FIGURE 4. RVA PROFILES OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.



SOURCE: The author (2018).

The curves for the analysed samples confirmed that the *Disocorea sp.* native starch sample (M1) was the only sample that presented a very different profile from the others. The M1 sample showed an intense viscosity peak at ≅ 84 ºC and a strong tendency to retrogradation ≅ 3,000 mPa.s. Similar curve profiles were obtained by previous studies of *Dioscoereaceas* starches from *D. sp.* and other species (JAYAKODY et al., 2007b; ZAIDUL et al., 2007; JIANG et al., 2012; OKUNLOLA; AKINGBALA, 2013; HORNUNG et al., 2017).

TABLE 4. RVA RESULTS OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.

Samples	Pasting temperature /°C	Viscosity peak /mPa.s	Peak time /sec	Setback /mPa.s	Breakdown /mPa.s	Final viscosity /mPa.s
M1	78.6 ± 0.3^{e}	2,392 ± 2.1^{a}	566 ± 0.1^{d}	1,340 ± 0.7^{a}	632 ± 4.9^{a}	3,105 ± 1.4^{a}
M2	85.3 ± 0.2^{bc}	666 ± 1.4^{h}	764 ± 0.2^{abc}	207 ± 4.2^{i}	2.5 ± 0.7^{d}	878 ± 3.5^{j}
M3	93.6 ± 2.3^{a}	722 ± 2.1^{g}	780 ± 0.1^{ab}	212 ± 4.2^{i}	1.5 ± 0.7^{d}	938 ± 2.8^{h}
M4	81.00 ± 0.2^{de}	964 ± 2.1^{d}	726 ± 0.1^{c}	533 ± 4.9^{d}	9.5 ± 1.4^{c}	1,491 ± 1.4^{d}
M5	79.7 ± 0.3^{e}	1,117 ± 2.8^{b}	778 ± 0.1^{ab}	611 ± 0.7^{c}	5.5 ± 0.7^{cd}	1,722 ± 1.4^{c}
M6	93.2 ± 1.7^{a}	656 ± 2.1^{i}	780 ± 0.1^{ab}	238 ± 0.7^{h}	1.5 ± 0.7^{d}	898 ± 0.7^{i}
M7	82.8 ± 0.1^{cd}	935 ± 3.5^{f}	780 ± 0.1^{ab}	449 ± 1.4^{f}	1.5 ± 0.7^{d}	1,385 ± 0.7^{f}
M8	80.00 ± 0.1^{e}	1,101 ± 1.4^{c}	752 ± 0.5^{bc}	813 ± 4.2^{b}	29.5 ± 0.7^{b}	1,883 ± 3.5^{b}
M9	87.2 ± 1.1^{b}	964 ± 1.4^{d}	776 ± 0.1^{ab}	490 ± 0.7^{e}	1.5 ± 0.7^{d}	1,455 ± 0.7^{e}
M10	87.1 ± 2.0^{b}	957 ± 2.1^{e}	810 ± 0.7^{a}	397 ± 1.4^{g}	5.5 ± 0.7^{cd}	1,351 ± 1.4^{g}

mPa.s “millipascal-second”, sec “seconds”. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).
SOURCE: The author (2018).

Viscosity peak values are attributed to the swelling of starch granules, and this is related to the onset of gelatinisation (pasting temperature), which can be compared to the onset temperatures (*To*) obtained by DSC analysis. Comparing the pasting temperatures (RVA) with the *To* (DSC) values that were obtained it was verified that the RVA onset temperatures were higher for all the samples that were analysed. This discrepancy was attributed to the fact that this technique is less sensitive in detecting when the disruption of starch starts compared to the DSC technique (OKUNLOLA; AKINGBALA, 2013; HORNUNG et al., 2017).

The highest values for viscosity peak and final viscosity were found for the M1 sample (100 % of *D sp.* starch – 2,4 mPa.s) and for the samples that contained *D sp.* starch in their mixture (M4; M5; M7 to M10): the higher the proportion, the higher the values. The amylose ratio is a major factor that can affect starch pasting properties (HUANG et al., 2015). It is assumed that starches with low amylose content have low viscosity (viscosity peak, final viscosity) although damaged starches leach out amylose and amylopectin, which increases the starch paste viscosity (CAI et al., 2014).

Small granules gelatinise less than larger ones. The peak time is the time when a viscosity peak occurs, and it is inversely relative to the sensitivity of starch to heat (OKUNLOLA; ODEKU, 2011). Apart from M1, the samples did not present

significant differences between their results, as can be observed for the SEM - Average size (Table 3) results. For the breakdown parameter, it was found that samples M1 (native *D. sp.* starch) and M8 (70/15/15 % mixture of *D. sp.*; *D. piperifolia* and *D. trífida*) showed the highest significant differences between the samples. Once again, the *D. sp.* starch proportion influenced this property, as for viscosity peak and final viscosity.

The setback is generally related to the retrogradation (gelling ability) tendency of starch pastes. It represents the viscosity increase due to the rearrangement of amylose molecules during cooling (HUANG et al., 2015). As expected, the *Dioscorea sp.* native starch showed the highest apparent amylose content. Samples M1 (100 % *D. sp.*), M4 (50/50 % of *D. sp* and *D. piperifolia*), M5 (50/50 % *D. sp.* and *D. trífida*) and M8 (70/15/15 % *D. sp*; *D. piperifolia* and *D. trífida*) presented the highest tendency to retrogradation (setback results – Table 4). The lowest results were obtained for the native starches of *D. piperifolia* and *D. trífida*, and for the M6 mixture with no *D. sp.* content (50/50 % of *D. piperifolia* and *D. trífida*). The study of starch's tendency to retrogradation (setback results on RVA) is very important in order to assess the industrial applications of starches. Usually, the tendency to retrogradation is considered undesirable due to the staling of bread and other starch-rich foods, which causes low shelf life (WANG et al., 2015). However, starch paste retrogradation is required in some applications, such as breakfast cereals, parboiled rice and dehydrated mashed potatoes, to provide a slow enzymatic digestion that produces a moderated release of glucose into the blood stream, which is preferable in nutritional terms (COLLAR; ROSELL, 2013; WANG et al., 2015).

Starch pasting properties are also affected by the starch source, its amylose/amylopectin ratio, granule damage, granule swelling, granule size and rigidity (JIANG et al., 2012, 2013; HUANG et al., 2015; HORNUNG et al., 2016, 2017).

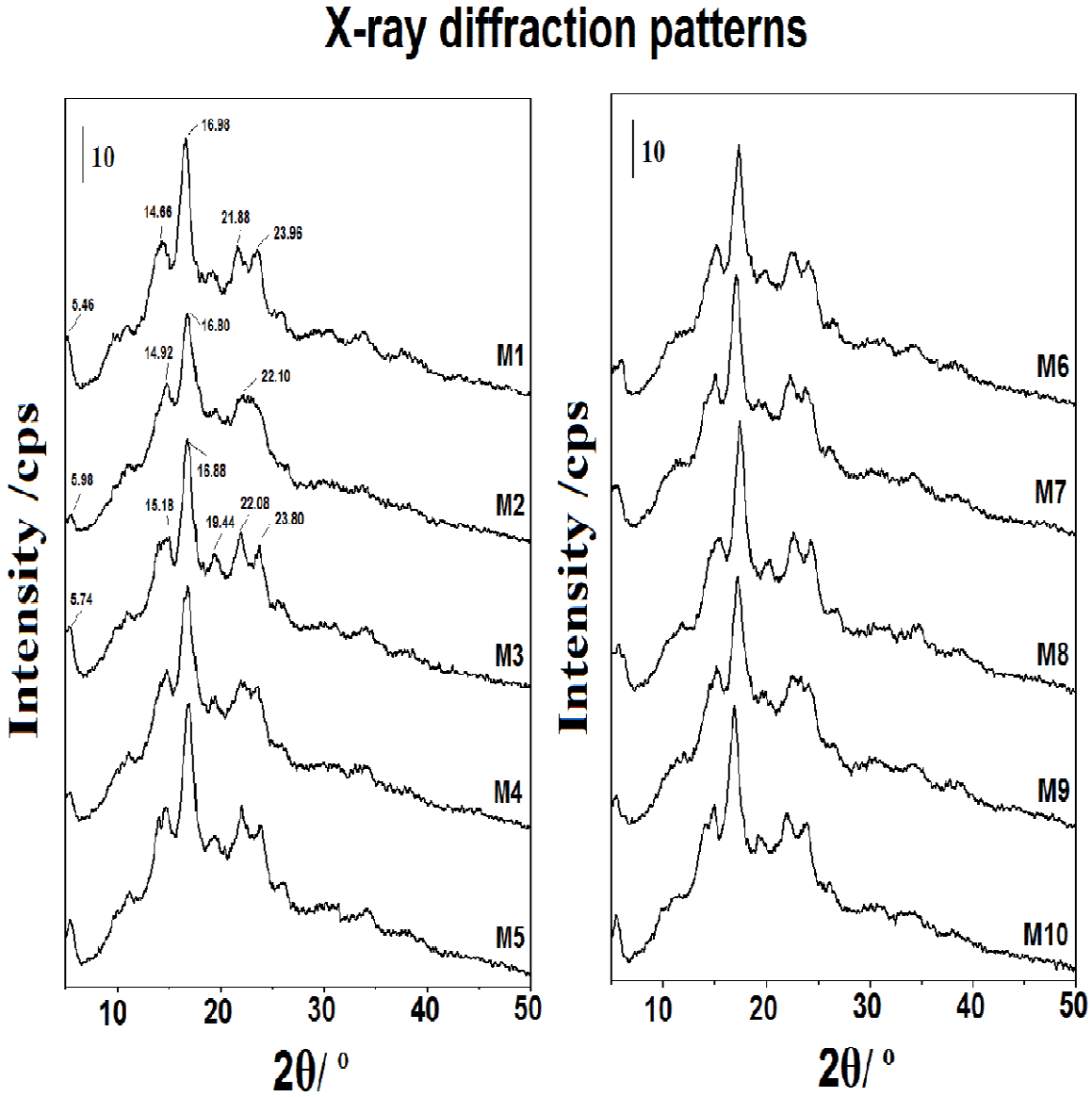
3.4 Morphological analysis

3.4.1 X-ray diffraction (XRD)

According to the XRD results (Table 3) the native starch samples M2 (*Dioscorea piperifolia*) 24.6 % and M3 (*Dioscorea trífida*) 25.4 % presented the

highest values for relative crystallinity (%), and sample M1 (*Dioscorea sp.*) 15.6 % presented the lowest values. This corroborated with the apparent amylose content analysis results (%) that were obtained, which were 12.5, 13.4 and 28.2 respectively. With the exception of sample M9, the mixed starch samples M4 to M8 and M10 showed significant different results for relative crystallinity compared with the native starch samples (M1 to M3), which demonstrates that the mixing process that was performed altered the relative crystallinity of the native starches. Previous studies have reported that the percentages of starch crystallinity increase in line with a decrease in the amylose content (CHEETHAM; TAO, 1998; SASAKI; YASUI; MATSUKI, 2000; LAWAL; ADEBOWALE; ODERLINDE, 2004; KUAKPETOON; WANG, 2006; JIANG et al., 2012; HUANG et al., 2015). These differences have been attributed to factors such as the botanical source, starch composition, moisture content and treatment of samples (JIANG et al., 2013).

FIGURE 5. XRD DIFRATOGRAMS OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.



SOURCE: The author (2018).

Native starch granules are semi-crystalline materials that can present three distinct diffraction patterns (Types: A - 15, 17, 18, 20 and 22º into 2*θ*; B – 5, 6, 14, 17, 18, 19 and 23º into 2*θ*; and C – a mixture of the reflection angles from types A and B) (JAYAKODY et al., 2007b; HORNUNG et al., 2015, 2016, 2017). X-ray diffraction detects long-range ordered structures involving regular and repeated arrangement of double helices, thereby reflecting the three-dimensional order of starch crystallinity (WANG et al., 2015).

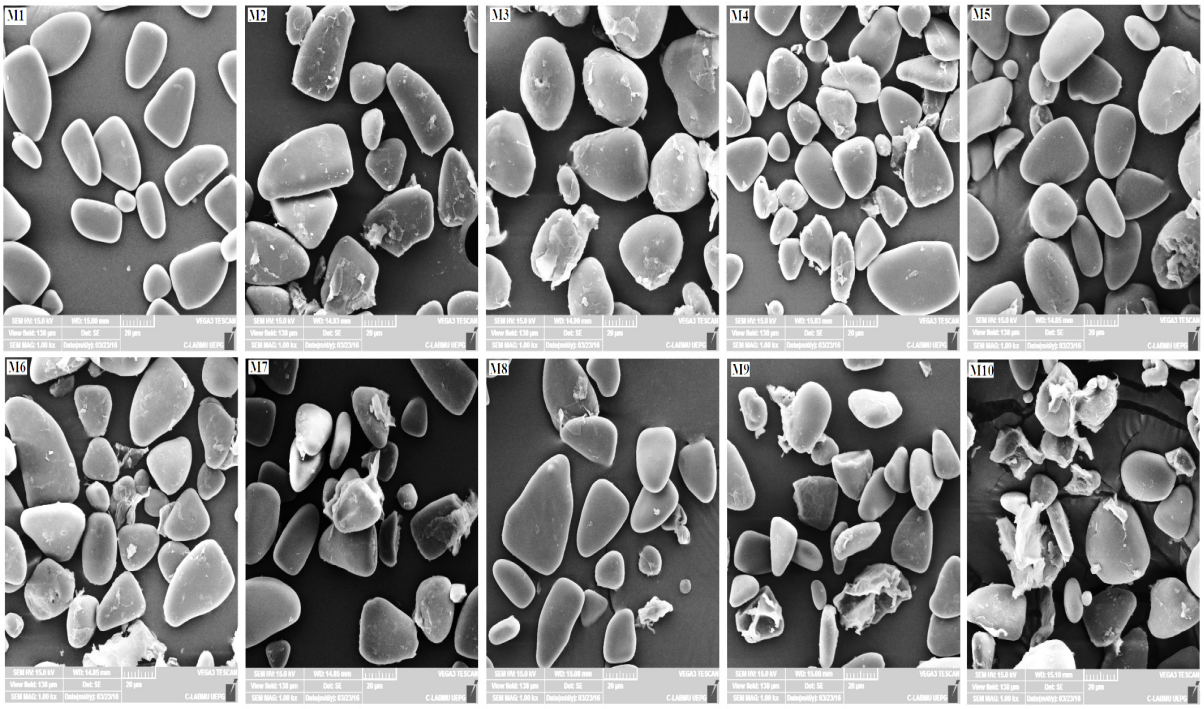
The XRD patterns (Fig. 5) show that the native starch samples presented reflections centered at 5, 14, 15, 16, 19, 21, 22 and 23º at 2*θ* angles, which are characteristic of 'C'-type starches, an intermediate crystalline form which is believed

to be a composite of type A and B starches (RILEY et al., 2008). This was expected for the tuber starch of this species (JAYAKODY et al., 2005, 2007b; CONFORTI; LUPANO, 2007; GARRIDO et al., 2012; HORNUNG et al., 2017). The mixture samples M8, M9 and M10 showed the reflections as the proportional content of each native starch. In terms of the other mixture samples (M4, M5 and M7), it was observed that the *D. sp.* starch reflection doublet at ≅ 22 to 23 º (2θ) angles predominated. The same was observed for the M6 sample, which presented the *D. trífida* doublet. XRD patterns are also related to the tendency to retrogradation of starch. In most cases 'B' type pattern starches are retrograde (WANG et al., 2015), as was found for the studied native starch *Dioscorea sp.* (M1), Table 4, and for the mixtures that contained *Dioscorea sp.* (M4, M5, M7, M8, M9 and M10). The retrogradation process is related to the amylose content, and the degree of relative crystallinity is used as a measurement of apparent amylose content (TESTER; KARKALAS; QI, 2004; STAWSKI, 2008). The results obtained in the present study for apparent amylose content confirmed its relationship to the tendency to retrogradation (setback – Table 4) and to the degree of relative crystallinity (Table 3). It also relevant to mention that starches with a low degree of relative crystallinity, which is related to the amylose content, show increased enthalpy for gelatinisation, as was verified for sample M1 (*D. sp.* native starch) (CHUNG; CHANG; LIM, 2004). Similar results for XRD patterns and for the degree of relative crystallinity have been found for the yam tubers of different varieties of the *Dioscorea* species (JAYAKODY et al., 2007b, 2007a; YUAN et al., 2007; RILEY et al., 2008; PÉREZ et al., 2011; JIANG et al., 2012, 2013; ZHANG et al., 2012; HORNUNG et al., 2017).

3.4.2 Morphological profile of granules

In this study, SEM micrographs were performed to analyse the granular morphology and average size of the granules. The micrographs are shown in Fig. 6 and the average size and granule size range are presented in Table 3.

FIGURE 6. SEM IMAGES (1000X) OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.



SOURCE: The author (2018).

Fig. 6 shows that the native starch samples M1 (*D. sp.*), M2 (*D. piperifolia*) and M3 (*D. trífida*) presented different granular sizes and forms. The mixed samples presented granular forms and sizes that varied according to the proportion of native starch content.

The *Dioscorea sp.* native starch (M1) sample showed some oval shapes but they were predominantly triangular with a smooth surface. The starch sample M2 showed the largest granules (37.7 µm) and widest size range (17 to 57 µm) of the native starches that were studied and it contained flaky, oblong-triangular and oval-triangular forms. In the *Dioscorea trífida* native starch sample (M3), oval shapes predominated and its average size and size range were similar to the results for sample M2. From the micrographs of the mixed starch samples it was verified that even smaller granules were registered and that the average size and size range obtained were lower than for the native starch samples. The morphology and size of starch granules depends on the biosynthesis of starch, physiology of the plant, botanical origin and amylose content (DEEPIKA; JAYARAM KUMAR; ANIMA, 2013; LAN et al., 2015). Similar results were found in previous studies (JAYAKODY et al., 2007b; JIANG et al., 2012; FALADE; AYETIGBO, 2015; HUANG et al., 2015; LAN et al., 2015; ÖZVURAL; HUANG; CHIKINDAS, 2016; HORNUNG et al., 2017).

To have a better understanding of the functional properties of starches it is important to determine their shape and size (KOKSEL et al., 2008; HORNUNG et al., 2017). Starches with a wide range, such as samples in the present study, are suitable for the pharmaceutical industry; they perform better in terms of influencing the density of tableting or in filling gelatin capsules. For food products, the diameter of granules is an important parameter regarding particle-particle interactions, mixing, and homogeneity for formulation (RILEY et al., 2008; FALADE; CHRISTOPHER, 2015). In their study of the physicochemical and structural properties of maize and potato starches as a function of granular size, (DHITAL et al., 2011), showed that granular size had a strong positive correlation to the relative degree of crystallinity. They verified that starches composed of large granules had a high degree of relative crystallinity and slightly decreased T_p and T_c gelatinisation temperatures, as was found for samples M2 and M3 in the present study Table 3.

3.4.3 Colour analysis

The colour of food ingredients is essential for the food industry as an attribute of food quality (freshness, maturity, variety and desirability) and also consumer acceptance (WU; SUN, 2013). The CIE 1976, or as it is more commonly named, the CIELAB (*L**; *a**, *b**) colour space, is the most widely used system for food due to its uniform perception of the colour and its perception, which is close to that of the human eye (LEÓN et al., 2006; QUEVEDO et al., 2009; WU; SUN, 2013). Three independent coordinate axes obtain information regarding the three colour attributes of lightness, hue and chroma. The *L** represents lightness, with values ranging from 0 (black) to 100 (white), and parameters *a** (from - green to + red) and *b** (from - blue to + yellow), with values from -120 to 120 (LEÓN et al., 2006; HORNUNG et al., 2015, 2016, 2017).

Visually, the native starches from *D. sp* (M1), *D. piperifolia* (M2) and *D. trífida* (M3) were white, light yellow and pale purple respectively, and in the mixtures (M4 and M6 to M10) where *D. piperifolia* native starch was present, a light yellow colour predominated. The CIELAB colour results for the studied samples are shown in Table 5.

TABLE 5. COLORIMETRY RESULTS OF: NATIVE SAMPLES: M1 – *D. SP.*; M2 – *D. PIPERIFOLIA*; M3 – *D. TRÍFIDA*; MIXTURES: M4; M5; M6; M7; M8; M9; M10.

Samples	Colour Results		
	L*	***a****	***b****
M1	94.00 ± 0.3^{a}	1.00 ± 0.0^{g}	3.7 ± 0.1^{i}
M2	71.1 ± 0.8^{h}	11.6 ± 0.1^{a}	36.6 ± 0.1^{a}
M3	73.2 ± 0.6^{g}	5.8 ± 0.1^{d}	5.0 ± 0.1^{g}
M4	77.3 ± 0.3^{de}	8.5 ± 0.5^{b}	29.00 ± 0.6^{c}
M5	81.00 ± 0.5^{c}	4.3 ± 0.1^{f}	4.5 ± 0.1^{h}
M6	73.4 ± 1.3^{g}	8.2 ± 0.1^{b}	23.4 ± 0.2^{d}
M7	78.00 ± 0.7^{d}	7.00 ± 0.1^{c}	19.7 ± 0.3^{e}
M8	84.4 ± 1.0^{b}	5.00 ± 0.1^{e}	15.8 ± 0.1^{f}
M9	75.00 ± 1.0^{f}	8.5 ± 0.2^{b}	31.1 ± 0.6^{b}
M10	76.00 ± 1.3^{ef}	6.1 ± 0.1^{d}	15.4 ± 0.3^{f}

Means followed by the same letter do not differ significantly by the Duncan test ($p < 0.05$).
SOURCE: The author (2018).

The colour results showed that the values for the *a** and *b** parameters were significantly different between all the samples. The *L** values also showed significant differences between the samples, except for between samples M4 and M7; M4 and M10; and M9 and M10. As expected, sample M1 presented the highest lightness result (94.00 *L**) and the lowest values for *a** (+ 1.00) hue and *b** (+ 3.7) chroma. The samples composed of more than 50 % of *D. sp.* starch showed high values for lightness (*L**: M5 81.00; M8 84.4). Although sample M8 presented a higher *L** value than M5, it presented a higher *b** value due to the *D. piperifolia* native starch content. The *D. piperifolia* native starch sample (M2) had the lowest value for lightness (71.1) and the highest values for *a** (+ 11.6) tendency to red and for *b** (+ 36.6) strong tendency to yellow. This tendency was found for all the mixed samples which contained *D. piperifolia* native starch (M4 and M6 to M10). The *D. piperifolia* Humb. var. Wild tuber contains pigments such as carotenoids that may be carried over to the starch, which could explain its colour property (RODRIGUES et al., 2012; HORNUNG et al., 2017). A slightly higher value for lightness was found for the sample M3 (*D. trífida*) starch compared to sample M2 and it was lower than sample M1. The *a** and *b** results were higher than for sample M1 and lower than for sample M2. Even at a concentration of 50 % and 70 % (sample M3), as in the case of samples M6 and M9, the colour tendency that predominated for the *a** and *b** results was as for sample M2.

Starches that show pigmentation can be applied in the formulation of products without having a negative impact on the colour characteristics of food products etc.

because the crucial factor is the amount that is used in the formulations (FALADE; CHRISTOPHER, 2015; HORNUNG et al., 2017).

4 Conclusions

The proportions applied enhanced the thermal stability for the mixed samples in a range up from 12 to 17 ºC (TG-DTA). Moreover, DSC presented a high decrease in the gelatinisation enthalpy due to the content of different starches. In RVA, it was noteworthy that there was a large decrease in the tendency to retrogradation of the *D. sp* native (M1: 1,340 mPa.s) starch after mixing (M4 –M5; M7 – M10: 533; 610; 499; 813; 490; 397 mPa.s, respectively). The average size (SEM) of the starch granules and the degree of relative crystallinity (XRD) varied widely after mixing (SEM 6 to 57 µm; XRD 12 to 19 %). The colour analysis showed that in a proportion of 70% of the *D. sp* and *D. trífida* native starches (samples M8 and M10) the *D. piperifolia* tendency to yellow decreased considerably. From the characteristics displayed, the mixture process suggests a positive outlook for modifying the functional properties of *Dioscorea sp*., *Dioscorea piperifolia* and *Dioscorea trífida* native starches.

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CHAPTER 4

GREEN DEVELOPMENT OF BIODEGRADABLE FILMS BASED ON NATIVE YAM (DIOSCOREACEAE) STARCH MIXTURES

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Green development of biodegradable films based on native yam (Dioscoreaceae) starch mixtures

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Abstract

The mixture of starch comprising starches from different botanical origins and species can improve the mechanical properties of films and coatings. Thus, the aim of this work was to develop a sustainable process of starch modification to obtain enhanced starch films through mixing three *Dioscoreaceae* starches and to study the films resultant mechanical (tensile strength and elongation at break), thermal (glass transition and melting temperature) and physicochemical (moisture, solubility, thickness, colour, transparency, light transmission, water vapour permeability, crystallinity, and surface uniformity) properties. The films obtained after the mixing process showed low moisture content and high transparency, high solubility desirable for biodegradability, and significantly different thickness. An improved light barrier was achieved and water vapor permeability barrier properties were obtained. Using differential scanning calorimetry, it was observed that the glass transition temperature of the films decreased. The starch mixture improved the mechanical characteristics of the films by 200 % for tensile strength and 232 % for elongation at break. After mixing, the films showed increased crystallinity and no crack or pinholes in their surface. These findings demonstrate that the yam starch mixtures formed

strong and flexible films suitable for various industrial products through a simple green process.

Keywords: *Dioscorea* starch; edible film; sustainable process; biopolymer.

1 Introduction

Traditional packing with petroleum based plastic takes several years to degrade causing serious pollution issues (CAZÓN et al., 2017). The environmental concerns and the crescent interest of the consumers for green products have provided an opportunity to focus on the development of biodegradable materials (MALI et al., 2010; MEDINA JARAMILLO et al., 2016). Accordingly to Fakhouri, Martelli, Caon, Velasco, & Mei (FAKHOURI et al., 2015) starch can replace most of the polymers of petrochemical origin in the packing industry and reduce costs due to its widespread availability.

Starch films are entirely biodegradable, readily cast into films, easy to handle and often edible with no negative impact to the environment (FALGUERA et al., 2011; CHIUMARELLI; HUBINGER, 2012; DE MORAES CRIZEL et al., 2016). Biodegradable films can be obtained from a variety of native polymers such as starch, cellulose, collagen, chitosan, gelatin and others (SKURTYS et al., 2001; JIMÉNEZ et al., 2012; CAZÓN et al., 2017)(SKURTYS et al., 2001; JIMÉNEZ et al., 2012), from their mixtures or from isolated amylose and amylopectin components (MARQUES et al., 2006; ZAVAREZE et al., 2012; FAKHOURI et al., 2015; MEDINA JARAMILLO et al., 2015). For that reason, films can be obtained with materials which present thermoplastic properties implying that they can be thermally processed becoming melted or rubbery at a lower temperature than the decomposition temperature (XIE et al., 2010; ZHANG et al., 2013; MAHIEU; TERRIÉ; YOUSSEF, 2014).

Starch is the most applied material to develop biodegradable packings due to the advantages described above. Nevertheless, it presents technological challenges due to its hydrophilic character resulting in reduced mechanical (little flexibility and brittleness) and permeability properties (high water vapor and oxygen permeability) (BOURTOOM, 2008; ROJAS-GRAÜ; SOLIVA-FORTUNY; MARTÍN-BELLOSO, 2009; GHASEMLOU et al., 2013; EÇA; SARTORI; MENEGALLI, 2014). Therefore

native starch is blended with other polymers, different plasticizers or native starches from different botanical origins to enhance the mechanical and barrier properties mantaining the biodegradability (CAZÓN et al., 2017).

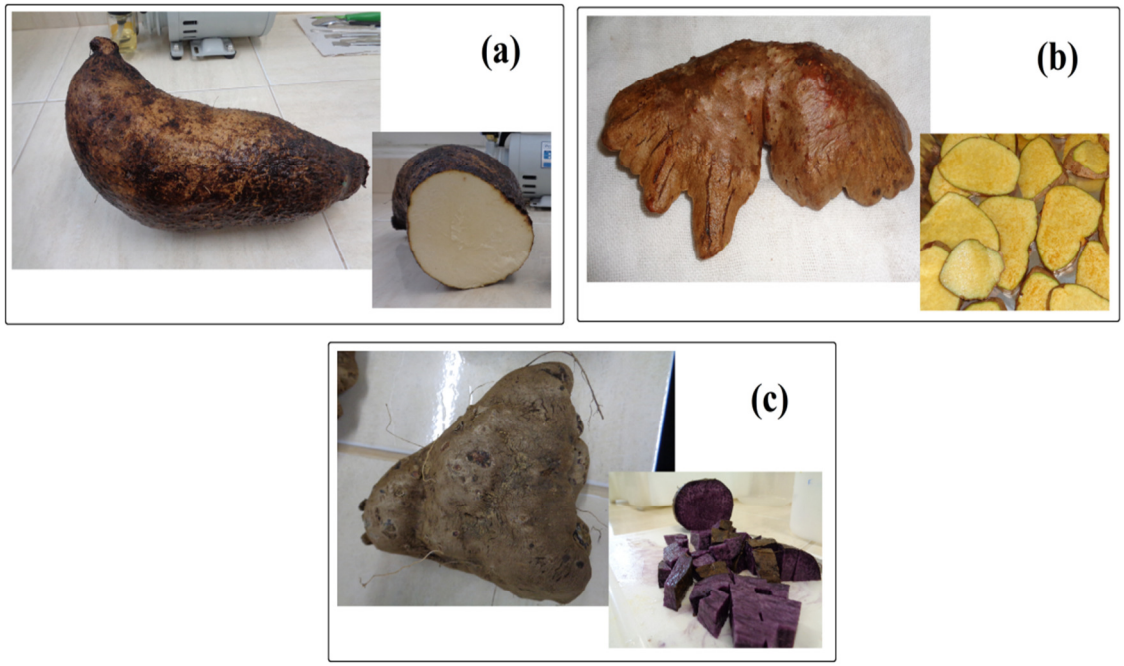
Yam tubers from the *Discoreaceae* genus are rich in starch comprising up to 70 % of the dry weight. Despite their economical value including being grown throughout tropical countries for food, pharmaceutical, and ornamental purposes with 85 % of the world yam production taking place in the West Africa, only limited literature is available (CORNET et al., 2014; HORNUNG et al., 2017c, 2017a). In Brazil, the *Dioscorea* tubers are mostly cultivated in the Amazon and Northeast regions and consumed as food staples (MENDES; SILVA; FAVERO, 2013; RAMOS et al., 2014). This crop is underutilized due to the lack of knowledge of its nutritional and functional properties and industrialization processes (HONGSPRABHAS et al., 2014; NASCIMENTO et al., 2015; ZHU, 2016). Therefore, application of the *Dioscorea* yams as raw materials for processed products could increase their agricultural demands contributing to the economic growth of developing countries such as Brazil (MEDINA JARAMILLO et al., 2015; PIÑEROS-HERNANDEZ et al., 2017). Some *Dioscorea* starches, as the one from *D.* sp species, are composed of amylopectin (about 70 %) and amylose (about 30 %), with the latter content being considered as ideal for developing starch films (LIPORACCI; MALI; GROSSMANN, 2005; POLYCARP et al., 2012).

Thus, the objectives of this work were to obtain and characterize the thermal and physicochemical properties of the tuber starch films and study the effects of the *Dioscoreaceae* native starches mixtures (*Dioscorea sp.*; *Dioscorea piperifolia* Humb. var. Wild and *Dioscorea trífida*) on their mechanical behavior.

2 Materials and methods

2.1 Materials

FIGURE 1. PHOTOGRAPHS OF YAMS: (A) *DIOSCOREA SP*. TUBERCLE, (B) *DIOSCOREA PIPERIFOLIA* HUMB. VAR. WILD TUBERCLE, AND (C) *DIOSCOREA TRÍFIDA* TUBERCLE.



SOURCE: The author (2018).

The native starches were obtained from three *Dioscoreaceae* tubers (Fig. 1.) from the species: *Dioscorea sp.*, *Dioscorea piperifolia* Humb. var. Wild. and *Dioscorea Trífida*, that were harvested in a crop area of Aripuanã city, Mato Grosso, Brazil (10º10'00" S / 59º27'34" W). The starch of each tuber was extracted and mixed as described in the previous group study (HORNUNG et al., 2017a).

2.2 Methods

2.2.1 Mixture design

A 3-component simplex centroid design (n = 7 assays) augmented with n = 3 central points to compose a total of 10 samples (Table 1) was applied. The performed design was processed with the STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA). The starch samples were coded as follows: 1, 2, 3 (*D. sp.*, *D.*

piperifolia and *D. Trífida*, respectively) native (unmixed) samples. The mixed starch samples were coded 4 to 10. Table 1 shows the starches concentration of the components in % and real values (g).

TABLE 1. CONTENT OF THE *DIOSCOREA* STARCHES USED IN THE STARCH MIXTURES FOR THE DEVELOPMENT OF THE FILM.

Samples	Components (%)			Components (g)		
	D. sp.	*D. piperifolia*	*D. trífida*	*D. sp.*	*D. piperifolia*	*D. trífida*
1	100	0	0	90	0	0
2	0	100	0	0	90	0
3	0	0	100	0	0	90
4	50	50	0	45	45	0
5	50	0	50	45	0	45
6	0	50	50	0	45	45
7	33	33	33	30	30	30
8	66	17	17	60	15	15
9	17	66	17	15	60	15
10	17	17	66	15	15	60

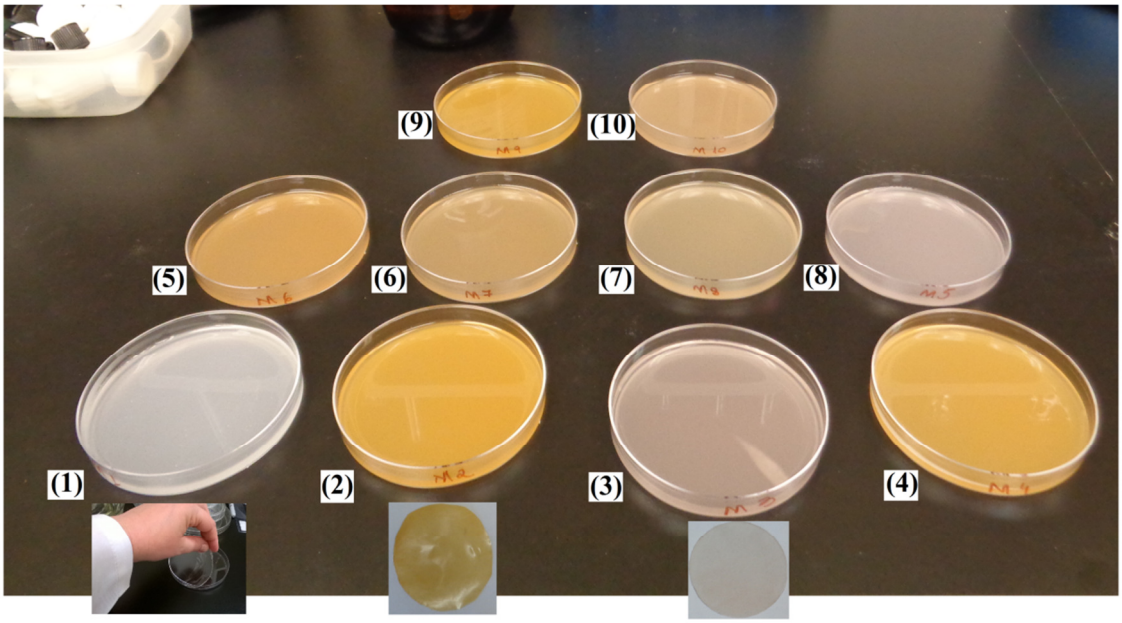
a) (*D.*) *Dioscorea*.

SOURCE: The author (2018).

2.2.2 Film production

To prepare the films, 0.88 g of each sample was mixed with glycerol (50 % of the starch mass or 0.44 g) and distilled water (20.68 g) to give a total solution weight of 22 g. The solution was heated to 90 ºC while stirring using a magnetic stirrer and left at this temperature for 3 minutes to allow complete gelatinization of the starch granules. The solution was cooled down to about 40 ºC and was cast on 91 mm diameter Petri acrylic dishes. The casted solutions (Fig. 2.) were dried in an air-circulating oven at 35 ºC for 18 hours before placing them in a desiccator with 43 % of relative humidity (K_2CO_3 - potassium carbonate saturated solution) to facilitate peeling and to equilibrate at room temperature for at least three days prior to analysis.

FIGURE 2. FILM CASTING SOLUTIONS AND FILM SAMPLES AFTER PEELING NATIVE STARCHES: (1) *D. SP.*, (2) *D. PIPERIFOLIA*, (3) *D. TRÍFIDA*, AND MIXTURES: (4), (5), (6), (7), (8), (9) AND (10).



SOURCE: The author (2018).

2.2.2.1 Starch apparent amylose content

The apparent amylose content of the native starches were carried out using a iodine colorimetric method, as previously described (MARTÍNEZ; CUEVAS, 1989).

2.2.3 Film characterization

2.2.3.1 Thickness

Film thickness was determined at 10 random positions with a digital micrometer, with a sensitivity of 0.001 mm (Mitutoyo – Absolute DIGIMATIC, Japan).

2.2.3.2 Moisture

The moisture content (%) of the films was determined gravimetrically in an air-circulation oven at 105 ºC for 24 hours. The moisture content was calculated considering the difference of the initial mass of the film and the final mass after drying (MARAN et al., 2013).

2.2.3.3 Solubility

The film solubility was measured as described by Maran et al. (MARAN et al., 2013) with some modifications: film discs of 2 cm diameter were cut, weighed and immersed in 50 mL distilled water in sealed beaker in constant shaking (200 rpm) at 25 ºC for 24 hours. The samples were dried in an air circulation oven at 105 ºC until constant mass. The solubility was calculated considering the dry sample mass before and after the test.

2.2.3.4 Transparency and light transmission

The film transparency and light transmission were measured as described by Zhang & Han (ZHANG; HAN, 2006) in a Genesys 10S UV-Vis (Thermo Scientific, USA) spectrophotometer at 600 nm.

2.2.3.5 Thermal properties

The glass transition temperature (T_g) and the melting temperature (T_m) of the films were determined using the differential scanning calorimeter (DSC Q200, TA Instruments) accordingly to the methodologies described earlier (CHUNG; CHANG; LIM, 2004; LUCHESE et al., 2015), with some modifications as follows: the films were cut into small pieces (4–5 mg) and placed in the aluminum DSC crucibles which were hermetically sealed prior to a DSC run using a heating rate of 10 ºC min^{-1} from -40 to 250 ºC, under nitrogen gas at a 50 mL min^{-1} flow rate. The mid-point temperature step-down shift in baseline was identified. The software used to calculate the glass transition temperature (T_g) and the melting temperature (T_m) of the films was the TA 60WS version 2.20 (Shimadzu Corporation, Japan).

2.2.3.6 Water vapor permeability (WVP)

The WVP was performed according to the methodology described recently (DE MORAES CRIZEL et al., 2016) with modifications: permeation cells were used consisting of glass tubes (height: 70 mm; diameter: 20 mm) filled with calcium chloride (30 g; 0 % RH – Relative Humidity). The tubes were sealed with the films,

weighed and placed in a desiccator containing a saturated solution of ammonium nitrate (providing a RH of 60 % at 25 ºC). The mass gain of films was determined by the difference between the mass at time zero and 72 hours. The results were determined using Eq. 1:

$$WVP = (w \times L) / (A \times t \times \Delta P) \quad \text{Equation 1}$$

Where "*w"* is the mass gain rate (water) (g) by the permeation cell, "*L"* is the thickness of the film (mm), "*A"* is the film permeation area (1.5 x 10^{-6} m^2), "*t"* is the time of diffusion (h), and "Δ*P*" is the differential vapor pressure of water through the film (Δ*P* = 3.282 kPa at 25 ºC).

2.2.3.7 Mechanical properties

The mechanical properties were determined according to the D882-91 method (ASTM, 1996) and the parameters analyzed were Tensile Strength (TS - MPa) and Elongation at Break (EB - %) performed in a Texture Analyser TA.XT Plus (Texture Technologies Corp., Hamilton, Canada). The method described earlier (GALDEANO et al., 2013) was used.

2.2.3.8 Structural analysis

The diffraction patterns of the native and the mixed starch samples and their derived starch films were obtained using an X-ray diffractometer (Rigaku, Ultima 4 model, Japan) and the degree of relative crystallinity was quantitatively estimated as described in the literature (HORNUNG et al., 2016; QIN et al., 2016). The starch samples were coded as: a, b, c (100 % of *D. sp.*, *D. piperifolia* and *D. Trífida* native starches, respectively) and the mixed starch samples were coded d to j following the same composition described in Table 1 for the starch films 4 to 10 components in % and grams (g).

The surface of the starch film samples were observed using a scanning electron microscope (SEM) (Tescan, VEGA 3, Kohoutovice, Czech Republic) under an acceleration voltage of 15 kV, a reading scale of 20 µm for magnification of 2000x

and 10 µm for magnification of 5000x after the samples had been coated with plasma of gold and palladium (BISINELLA et al., 2016; HORNUNG et al., 2016).

2.2.3.9 Color

Color determinations were performed in a Konica Minolta colorimeter (model CM-3500d, Minolta, CO., Japan) by using the 0.3 cm aperture port, dual 18-element silicon photodiode array with wedge-shaped continuous interference filter to record the CIELAB space (ROBERTSON, 1977).

2.2.4 Statistical processing

The Anderson-Darling's test was used to verify the normality of residuals (PEDRO; GRANATO; ROSSO, 2016) on all the data obtained for the film characterization. Data that did not follow a normal distribution were transformed by the Box–Cox technique. Subsequently, the homogeneity of variances was tested for all variables by the Brown–Forsythe test (Action Software version 2.5, Estatcamp, Campinas, Brazil). One-factor analysis of variance (ANOVA) was performed to determine significant differences among treatments, while the least significance difference Duncan's test was used to compare the means among treatments (GRANATO; DE ARAÚJO CALADO; JARVIS, 2014).

3 Results and Discussion

3.1 Starch apparent amylose content

The apparent amylose content (%) of the native *Dioscorea* starch were 26.9; 14.7; and 16.3 for the *D. sp.*; *D. piperifolia* and *D. trífida* samples, respectively. Similar values have been reported for *D. sp* (26.7 % and 23.6 %) (PÉREZ et al., 2013). The *D. piperifolia* starch had apparent amylose content comparable to values previously reported by our laboratory and others (HORNUNG et al., 2017a). However, different values (ranging from 1.4 to 12 %) were found for *D. trifida* in another study of three genotypes of *D. trífida* from the Venezuelan Amazon (PÉREZ et al., 2011).

3.2 Film characterization

TABLE 2. MOISTURE, SOLUBILITY, THICKNESS, TRANSPARENCY, LIGHT TRANSMISSION OF THE FILMS FROM NATIVE STARCHES: 1 – *D. SP.*, 2 – *D. PIPERIFOLIA*, 3 – *D. TRÍFIDA*, ANDSTARCH MIXTURES: 4, 5, 6, 7, 8, 9, 10.

Samples	Moisture	Solubility	Thickness	Transparency	Light Transmission
	(%)	(%)	(mm)	(A_{600}/mm)	%, T_{600}
1	11.16 ± 0.33	40.89 ± 1.21^{j}	0.10 ± 0.01^{c}	1.16 ± 0.01^{de}	16.19 ± 0.01^{c}
2	10.58 ± 0.88	47.10 ± 2.22^{d}	0.12 ± 0.01^{b}	1.83 ± 0.15^{a}	12.63 ± 1.40^{j}
3	10.47 ± 0.33	49.73 ± 1.63^{a}	0.12 ± 0.01^{b}	1.09 ± 0.01^{ef}	18.57 ± 0.01^{a}
4	10.70 ± 0.53	43.57 ± 2.92^{h}	0.12 ± 0.01^{b}	1.22 ± 0.03^{d}	14.45 ± 0.01^{h}
5	11.08 ± 1.31	45.87 ± 2.20^{f}	0.13 ± 0.02^{a}	1.22 ± 0.05^{d}	17.09 ± 0.56^{b}
6	11.03 ± 0.37	47.45 ± 1.78^{c}	0.12 ± 0.03^{b}	1.61 ± 0.01^{b}	14.91 ± 0.01^{f}
7	10.39 ± 0.84	45.96 ± 1.05^{e}	0.13 ± 0.02^{a}	1.21 ± 0.01^{d}	15.82 ± 0.01^{d}
8	10.65 ± 0.45	41.64 ± 1.48^{i}	0.11 ± 0.01^{bc}	1.03 ± 0.04^{f}	14.79 ± 0.49^{g}
9	10.53 ± 0.38	45.25 ± 1.28^{g}	0.13 ± 0.01^{a}	1.36 ± 0.03^{c}	13.09 ± 0.35^{i}
10	11.09 ± 0.45	48.15 ± 1.47^{b}	0.13 ± 0.01^{a}	1.22 ± 0.01^{d}	15.79 ± 0.01^{e}
p (normality)*	0.20	0.71	0.08	0.44	0.90
p (Levene)*	0.51	0.92	< 0.0001	< 0.0001	0.02
p (Anova)**	0.44	-	-	-	-
p (Kruskal-Wallis)***		< 0.001	< 0.03	< 0.01	< 0.001

a) (A_{600}) Absorbance in 600 nanometers; b) (T_{600}) Transmittance in 600 nanometers; c) (*) Probability values obtained by Anderson-Darling test for normality and by the Levene test for homogeneity of variances. d) (**) Probability values obtained by one-way ANOVA. e) (***). Probability values obtained by Kruskal–Wallis test. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).

SOURCE: The author (2018).

3.2.1 Thickness

The films showed significant differences ($p < 0.05$) as compared to the film obtained from the *Dioscorea sp.* native starch. The thickness measurements recorded for the films obtained from the native starches *D. piperifolia* (sample 2) and *D. trífida* (sample 3) showed that they were significantly different from samples 5, 7, 9 and 10. Gutiérrez, Tapia, Pérez, & Famá (GUTIÉRREZ et al., 2015a) working with edible films made from native and modified yam (*D. trífida*) and cassava starch, suggested that the *D. trífida* starch has a high interaction with the plasticizer (glycerol) during the gelatinization that could result in thicker films. Following gelatinization, the crystalline molecules present may act as nuclei for recrystallization of amylaceous macromolecules during cooling and storage, producing brittle film (GARCÍA; MARTINO; ZARITZKY, 2000; GUTIÉRREZ et al., 2015a). Plasticizers can

limit the polymer chain alignment (recrystalization and crystal growth) by maintaining the water molecules in the starch gel network and lowering reassociation of the amylose and amylopectin molecules (DONHOWE; FENNEMA, 1993) hence avoiding cracks and pinholes in the starch film surface.

3.2.2 Moisture

The importance of keeping proper moisture control of the starch films relies on the fact that its content can ensure adequate flexibility (Elongation at Break), resiliency (Tensile Strength) and also influence solubility and water vapor permeability due to the hydrophilicity of the hydroxyl groups (SHAH et al., 2016). It can be seen (Table 2) that the films displayed a range from 10.39 % (w/w) (sample 7) to 11.16 % (sample 1) of moisture, which is considered as film stable against microbial growth and ability to maintain a continuous matrix for longer periods (SOTHORNVIT; RODSAMRAN, 2008). This moisture content is also influenced by the percentage of glycerol (plasticization and humectant effect) utilized in the development of the film (MALI et al., 2005; JIMÉNEZ et al., 2012).

3.2.3 Solubility

The solubility of polymer-based films directly impacts its biodegradability and contributes to the decision of their best applicability (MARAN et al., 2013; MEDINA JARAMILLO et al., 2015). For example, films considered of high solubility (> 60 %) in water at room temperature, can be applied to phytochemical or pharmaceutical drug encapsulation where higher solubility is desired (SPADA et al., 2012, 2013; GHASEMLOU et al., 2013) and for semi-finished foods (preparation under cooking) (FAKHOURI et al., 2007; ZAVAREZE et al., 2012); films with solubility < 50 %, such as the ones obtained in this study (Table 2), can be applied to fruit covering, high-fat meat and fish safely protecting against water loss without losing its integrity (SOTHORNVIT; RODSAMRAN, 2008; BIERHALS; CHIUMARELLI; HUBINGER, 2011; MARAN et al., 2013; SÁNCHEZ-ORTEGA et al., 2016).

Values for solubility were significantly different ($p < 0.05$) (Table 2) ranging from 40 % - less soluble (sample 1 – film from *D. sp.* native starch) to 49 % - more soluble (sample 2 – film from *D. piperifolia* native starch). The samples that

presented more solubility contained more than 17 % of the *D. piperifolia* (samples: 2; 4; 6; 7 and 9) and *D. trífida* (samples: 3; 5; 6; 7 and 10) native starches. Solubility differences could also be attributed to the amount of glycerol used and its interaction with the starch which in turn is influenced by characteristics including botanical origin, constituents (e.g. amylose and amylopectin ratio, protein and lipid content), rheological and gelatinization properties (JIMÉNEZ et al., 2012; PALMA-RODRÍGUEZ et al., 2012; XIE et al., 2014).

3.2.4 Transparency and light transmission

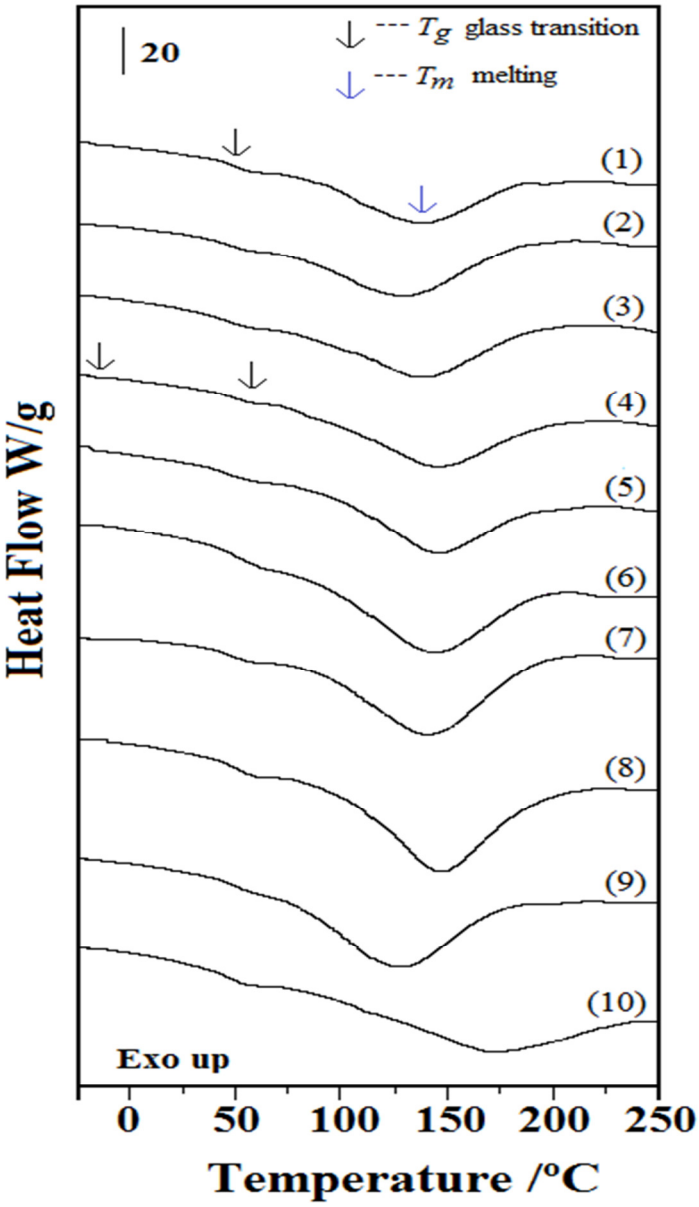
Optical properties are parameters of prime importance for films since there are mostly used for food packaging (MALI et al., 2004; FAKHOURI et al., 2007; DE MORAES CRIZEL et al., 2016). Sample 2 (*D. piperifolia* native starch film), Table 2, showed higher transparency (1.83 mm at A_{600}) and the lowest light transmission rate (12.63 %), and this was apparent in the films of the samples (4; 6; 9) with at least 50 % of this starch in its constitution. Previous studies about rice starch-based coating (DAS; DUTTA; MAHANTA, 2013), antimicrobial starch-based films (SALLEH; MUHAMAD; KHAIRUDDIN, 2009) and films from cellulose acetate and polystyrene (MEENAKSHI et al., 2002) describes that the light transmission of films are directly correlated to the particle size, where large particles in the film matrix can act as a barrier to light. One of the characteristics of the *D. piperifolia* native starch is its wide range of granular size ranging from 17 to 57 µm, when compared to the *D. sp* (17 – 43 µm) and *D. trífida* (18 – 44 µm) native starches (HORNUNG et al., 2017a). These findings could indicate a potential application of the films obtained from the *D. piperifolia* native starch (sample 2) and the mixtures with the *D. sp* and *D. trífida* samples (4, 6 and 9), as food packages that prevent oxidation degradation triggered by light transmission.

3.2.5 Thermal properties

Glass transition temperature (T_g) and melting temperature (T_m) are important parameters in evaluating the performance (mechanical properties, permeability, storage, shelf life) of polymer materials such as films and coatings (LI et al., 2011). The T_g is the temperature related to the moment that the amorphous regions of a

polymer changes from the “glassy” to a “rubbery” state (softening point), while the melting point or the T_m (above T_g) represents the transition between a crystalline solid (retrograded amylopectin molecules) and a liquid (limiting the softening point) (FALGUERA et al., 2011; JIMÉNEZ et al., 2012; ZHU, 2015).

FIGURE. 3. THERMAL PROPERTIES OF THE FILM SAMPLES FROM NATIVE STARCHES: (1) *D. SP.*, (2) *D. PIPERIFOLIA*, (3) *D. TRÍFIDA*, AND MIXTURES: (4), (5), (6), (7), (8), (9) AND (10).



SOURCE: The author (2018).

All film samples from the mixed starches showed different thermal profiles from the ones obtained on the native starches (Fig. 3.). Starch mixing altered the thermal characteristics of the native starch films. The curves also indicated one T_g event (shown with an arrow in Fig. 3. on the first sample to guide visualization).

When only one T_g is obtained for blended polymers in a DSC scan (as for samples 1 to 3, 9 and 10), it is generally an indication of the proper compatibility of their components (ARVANITOYANNIS; BILIADERIS, 1998; SU et al., 2010; AL-HASSAN; NORZIAH, 2012). However, when the starch films show two T_g events as for samples 4 to 8, it has been established in the literature that starch-glycerol films are partially miscible than the ones composed of starch-rich phases and glycerol-rich phases (KAR; PANDEY; RANA, 2015). In particular, phase separation of glycerol in temperatures below 0 °C, when glycerol concentration was over 30%, has been reported to occur in films which can be seen from two separate transitions in the DSC thermograms (FORSSELL et al., 1997; MATHEW; DUFRESNE, 2002; LIPSON; SCOTT, 2007), as observed in this study. The calculated differences in thermal parameters are shown in Table 3.

TABLE 3. THERMAL PROPERTIES AND WATER VAPOR PERMEABILITY OF THE FILMS FROM NATIVE STARCHES: 1 – *D. SP.*, 2 – *D. PIPERIFOLIA*, 3 – *D. TRÍFIDA*, AND STARCH MIXTURES: 4, 5, 6, 7, 8, 9, 10.

Samples	**DSC Results**		**Water vapor permeability**
	T_g / ºC	T_m / ºC	*WVP (g mm m^{-2}/ h kPa)*
1	52.06 ± 0.05^{a}	138.71 ± 0.01^{h}	1.22 ± 0.01^{a}
2	51.91 ± 0.28^{a}	132.29 ± 0.22^{i}	1.17 ± 0.01^{c}
3	48.18 ± 0.04^{c}	140.75 ± 0.01^{g}	1.15 ± 0.01^{i}
4	46.71 ± 0.01^{d}	142.96 ± 0.14^{d}	1.17 ± 0.01^{c}
5	43.72 ± 0.01^{e}	144.63 ± 0.18^{c}	1.16 ± 0.01^{d}
6	48.43 ± 0.12^{c}	141.54 ± 0.01^{e}	1.13 ± 0.01^{g}
7	41.25 ± 0.03^{f}	140.99 ± 0.01^{f}	1.15 ± 0.01^{e}
8	49.02 ± 0.01^{b}	145.70 ± 0.01^{b}	1.19 ± 0.01^{b}
9	48.18 ± 0.16^{c}	128.67 ± 0.34^{j}	1.14 ± 0.01^{f}
10	47.53 ± 0.05^{c}	169.92 ± 0.70^{a}	1.11 ± 0.01^{h}
p (normality)*	0.20	0.10	0.93
p (Levene)*	0.002	0.003	0.99
p (Anova)**	-	-	< 0.001
p (Kruskal Wallis)***	< 0.001	< 0.001	-

a) (T_g) glass transition temperature; b) (T_m) melting temperature; c) (WVP) Water vapor permeability. d) (*) Probability values obtained by Anderson-Darling test for normality and by the Levene test for homogeneity of variances. e) (**) Probability values obtained by one-way ANOVA. f) (***) Probability values obtained by Kruskal–Wallis test. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).

SOURCE: The author (2018).

The films developed with only native starches had glass transition temperatures of 48.18 ºC (*D. trífida* – sample 3), 51.91 (*D. piperifolia* – sample 2) to 52.06 ºC (*D. sp* – sample 1). Samples 1 and 2 were statistically similar to each other but significantly different from sample 3 and from samples 4 to 10. Since the same amount of glycerol (50 % of the starch mass) was used in the development of the film, the starch mixture used was able to shift downwards the glass transition temperatures (T_g) of the films. Reducing the T_g of starch films is important to prevent the formation of cracks and pinholes due to brittleness (MENZEL et al., 2013; XIE et al., 2015). Although it is important to mention that the T_g of the films cannot be lower than the storage temperature, or the starch recrystallizes (that is, amylose and amylopectin reassociation into crystalline segments) causing loss of flexibility due to rigidity (XIE et al., 2010, 2015; JIMÉNEZ et al., 2012). The films developed in this study, except for samples 5 and 7, could be applied to products that need or stay at temperatures below 46 ºC without thermal damages to the coating properties. On the contrary to the decrease which occurred in the T_g after mixing the starches, the melting temperature (T_m) was increased for the films obtained from the starch mixtures with values ranging from 140.99 ºC (sample 7) to 169.92 ºC (sample 10), except for sample 9. These observations could be due to the variable sizes of starch granules and to the transition of amylopectin microcrystallites (FREDRIKSSON et al., 1998; XIE et al., 2010; WANG et al., 2011) that composes the matrix of the film. In our previous work (HORNUNG et al., 2017a), the granules size range were determined. Corresponding size range of the granules are 17 – 43 *D. sp*; 17 – 57 *D. piperifolia* and 18 – 44 µm *D. trífida*. The moisture content of the films could also influence the melting temperature in addition to the amylose content, higher levels resulting in higher T_m (XIE; HALLEY; AVÉROUS, 2012; XIE et al., 2015). Sample 10 had the highest melting temperature (169.92 ºC) and the lowest moisture content (10.19 %).

3.2.6 Water vapor permeability (WVP)

For the application of the films to fresh or dehydrated food products, it is crucial to determine their water vapor permeability (WVP) (DE MORAES CRIZEL et al., 2016). Although starch based films present high technological features including easy degradability, environmentally friendly, low cost, low density, and excellent

resiliency (MALI et al., 2010; CHAMBI; GROSSO, 2011), these films have low vapor barrier properties. An alternative to enhance this characteristic is to test different formulations varying components concentrations (GUTIÉRREZ et al., 2015b, 2015a)

Table 3 displays the effects of the starch mixtures effects on the WVP properties of the resultant films. It can be seen that the films developed from the starch mixtures showed significant differences ($p < 0.05$) among them compared to the native starch films. The samples that presented the highest and lowest water vapor permeability among the non-mixed starches were the films developed with 100 % of *D. sp.* starch (1.22 g mm m^{-2}/ h kPa) and *D. trífida* starch (1.15 g mm m^{-2}/ h kPa), respectively. Although the starch films (samples 4 to 10) from the mixed samples showed lower WVP than the *D. sp.* starch film, samples 4 and 7 had similar values as the *D. piperifolia* and *D. trífida native* starch films. Low WVP values were recorded for samples 6 (1.13) and 10 (1.11) prepared with 45/45 g of *D. piperifolia* and *D. trífida native* starches and 15/15/60 g of *D. sp.*, *D. piperifolia* and *D. trífida native* starches, respectively. The results obtained are similar to the ones found by Muscat et al (MUSCAT et al., 2012) (1.26 g mm m^{-2}/ h kPa for the high-amylose starch film sample with 40% of glycerol) and Arabestani et al (ARABESTANI et al., 2016) (1.06 g mm m^{-2}/ h kPa for the bitter vetch protein-based film added of pomegranate juice in 10% and 50% of glycerol).

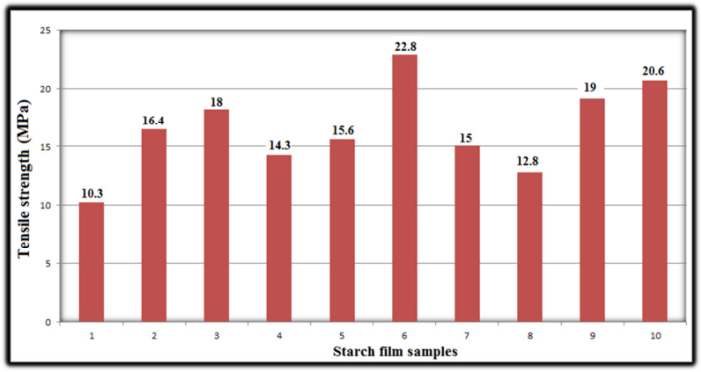
In spite of the fact that the mixing process did not enhance the Discoreaceae native starch films, it is essential to know the values of the WVP before applying the film to selected foods. These values determine the product type that can be packaged in the film, for example, fresh versus dehydrated foods (DE MORAES CRIZEL et al., 2016).

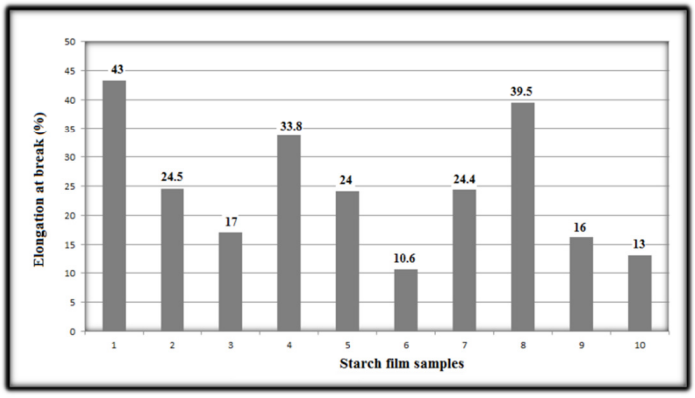
3.2.7 Mechanical properties

3.2.7.1 Tensile Strength (TS) and Elongation at Break (EB)

TABLE 4. MECHANICAL PROPERTIES OF THE FILMS FROM NATIVE STARCHES: 1 – *D. SP.*, 2 – *D. PIPERIFOLIA*, 3 – *D. TRÍFIDA*, AND STARCH MIXTURES: 4, 5, 6, 7, 8, 9, 10.

Samples	Mechanical Properties	
	TS (MPa)	EB (%)
1	10.28 ± 0.26^{h}	43.24 ± 0.12^{a}
2	16.45 ± 0.19^{d}	24.50 ± 0.02^{j}
3	18.21 ± 0.02^{c}	17.07 ± 0.03^{d}
4	14.28 ± 0.05^{f}	33.79 ± 0.20^{g}
5	15.61 ± 0.05^{e}	24.15 ± 0.05^{b}
6	22.85 ± 0.06^{a}	10.60 ± 0.40^{h}
7	15.09 ± 0.02^{e}	24.39 ± 0.20^{f}
8	12.78 ± 0.12^{g}	39.53 ± 0.07^{c}
9	19.11 ± 0.07^{b}	16.26 ± 0.28^{i}
10	20.64 ± 0.06^{b}	13.15 ± 0.07^{e}
p (normality)*	0.06	0.33
p (Levene)*	0.005	0.002
p (Kruskal Wallis)***	< 0.001	< 0.001





a) (TS) Tensile strength; b) (EB) Elongation at break. c) (*) Probability values obtained by Anderson-Darling test for normality and by the Levene test for homogeneity of variances. d) (**) Probability values obtained by one-way ANOVA. e) (***). Probability values obtained by Kruskal–Wallis test. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).

SOURCE: The author (2018).

Gelatinized starch granules can produce biodegradable films with mechanical properties that need further improvement in order to better protect food products. The use of mixture designs, as the one applied in this work (Simplex-Centroid), can provide the combination of components that gives the possibility of exploring how such components affect specific responses reducing variability, time for

development, overall costs, among other advantages (RAO; BARAL, 2011; DIAS et al., 2015).

Films used for packaging of foods or any other products should exhibit sufficient mechanical properties such as enough resistance to tensile strength and good flexibility to handle (elongation at break) (DE MORAES CRIZEL et al., 2016). Starch films developed with glycerol as a plasticizer showed lower results for tensile strength (TS) and higher values for elongation at break (EB) (Table 4). According to the literature (SKURTYS et al., 2001; CAZÓN et al., 2017), polymer films show TS values between 1 (collagen) and 140 MPa (gelatin) and EB values from 2.5 (alginate) to 205 % (cellulose); and starches, waxy maize (35 MPa), potato and tapioca (44 MPa) and wheat 46 MPa with 1.7, 3.1 and 2.9 %, respectively of EB.

The starch films developed in this work showed lower values for TS and higher results for EB compared to literature values. However, Arvanitoyannis & Biliaderis (ARVANITOYANNIS; BILIADERIS, 1998) found values similar to the ones obtained in this study of TS (ranging from 12 to 38 MPa) and EB (ranging from 2 to 29 %), for soluble starch mixed with different concentrations of methyl cellulose and glycerol. These differences could be attributed to the methods and handling applied, to the botanical origin of the starch, type and amount of plasticizer and the storage conditions of the films (SKURTYS et al., 2001; BOURTOOM, 2008; JIMÉNEZ et al., 2012; SHAH et al., 2016).

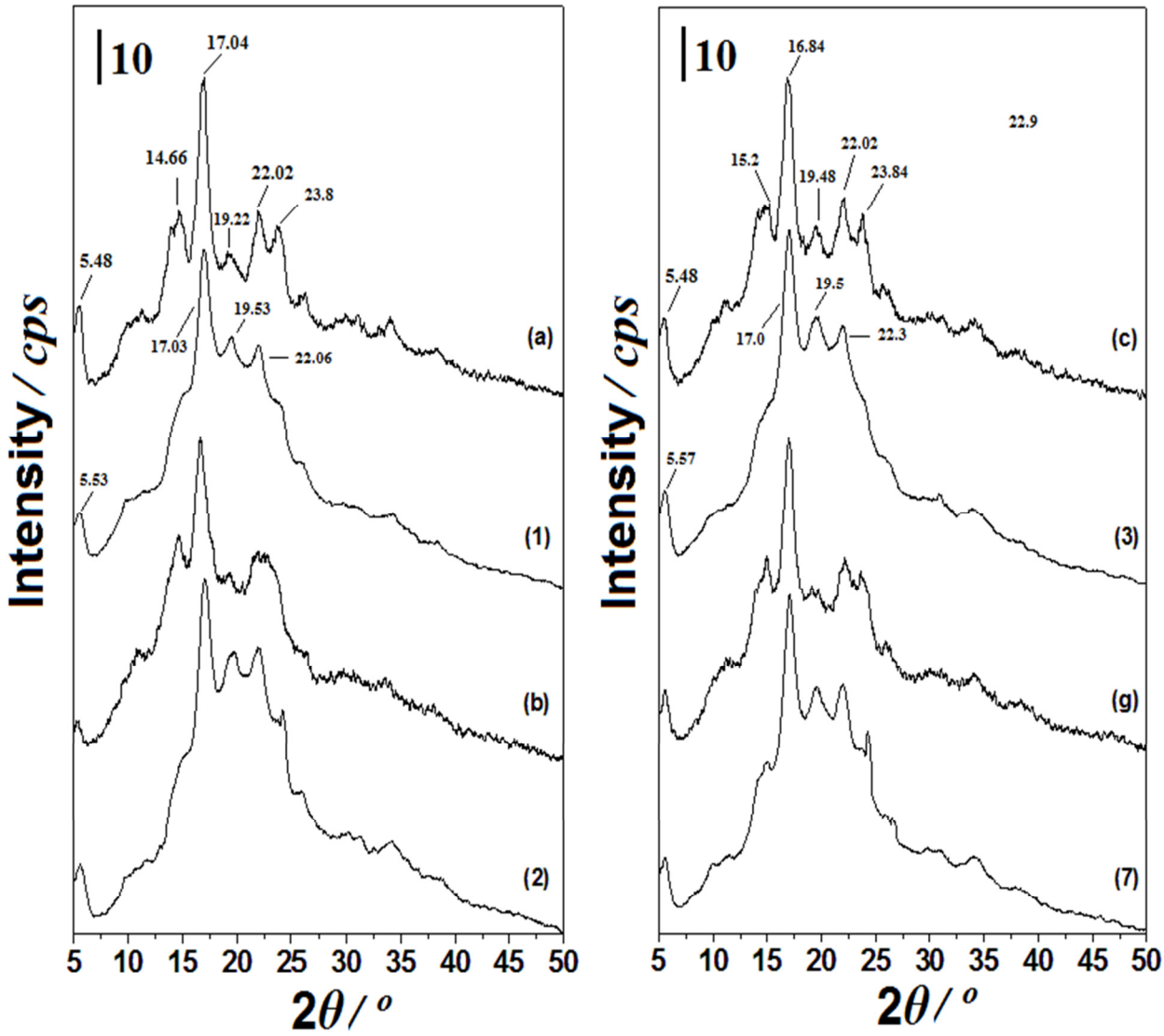
Samples 6, 9 and 10 that are mixtures containing increased concentrations of *D. piperifolia* and *D. trífida* native starches showed high values with their combination at 45g of each (samples 6) reaching the highest TS (22.85 MPa), that was an increase of 138 % compared to sample 2 (only *D. piperifolia*) and 125 % compared to sample 3 (only *D. trífida*). With respect to sample 10, the mixture proportion applied (17/17/66 % of *D.sp/D. piperifolia/D. trífida*, respectively), enhanced the mechanical resistance to tension (Tensile strength – Table 4) by 200 %. As a consequence of the TS decrease, the EB increases which is desirable for films, representing more flexibility and degradability (GHASEMLOU et al., 2013; LI et al., 2015; ARABESTANI et al., 2016; CAZÓN et al., 2017). Samples 6, 9 and 10 had lower EB values. Higher flexibilities (EB %) were shown by samples 1 (43 %), 4 (33 %) and 8 (39 %) that were developed with higher amounts of *D. sp.* native starch. For sample 8, the mixture proportion used enhanced the EB of sample 2 (only *D. piperifolia*) and sample 3 (only *D. trífida*) by 161 and 232 %, respectively.

3.2.8 Structural analysis

3.2.8.1 X-Ray Diffraction (XRD)

XRD was used to investigate the crystal structure of the native starches, starch mixtures and their derived film samples. The XRD patterns (Fig. 4) showed that the native starch samples (a – *D. sp*; b – *D. piperifoli*a; c – *D. trífida*) crystallographic patterns characteristic of 'C'-type starches centered at 5, 14, 15, 16.8, 17,18, 19, 22, 23 and 24° at 2*θ* angles (YANG; LU; HWANG, 2003; JAYAKODY et al., 2005; HORNUNG et al., 2017b, 2017c). Literature postulates that 'C'-type starches are an intermediate crystalline form that composes type A and B starches (YANG; LU; HWANG, 2003; JAYAKODY et al., 2005; HORNUNG et al., 2017b, 2017c). Similar results were reported for these tuber starch species (YANG; LU; HWANG, 2003; JAYAKODY et al., 2005; HORNUNG et al., 2017b, 2017c). However, the films presented crystallographic patterns centered at 5, 17, 19 and 22° at 2*θ* angles for the native starch-based film samples 1 (*D. sp*.) and 3 (*D. trífida*) in which angles 14, 15 and 24 existing in starches were not detected, and at 5, 17, 19, 22, 24 (24 was not detected in the native starch sample b - *D. piperifolia*) for the native starch-based film sample 2 (*D. piperifolia*) and for the starch mixture-based film sample 7 (composed of 33/33/33 % of *D. sp.*, *D. piperifolia* and *D. trífida*) also with an angle of 15 representing all the samples applied. Although an increase in the crystallinity was observed for the starch-based film samples 1-10, these angles are characteristic of amorphous materials (LI et al., 2015; MEDINA JARAMILLO et al., 2016) suggesting that the amount of glycerol used could had hindered the retrogradation process of the starch molecules (amylose and amylopectin) by affecting the chain attraction. Crystallinity values are displayed in Table 5.

FIGURE 4. XRD PATTERNS OF THE NATIVE STARCHES (A – *D. SP*; B – *D. PIPERIFOLIA* AND C – *D. TRÍFIDA*), NATIVE STARCH MIXTURE D (33/33/33 % OF *D. SP*/*D. PIPERIFOLIA*/*D. TRÍFIDA*) AND OF THEIR DERIVED STARCH-BASED FILMS (SAMPLES 1, 2, 3 AND 7).



SOURCE: The author (2018).

TABLE 5. XRD DIFFRACTOGRAMS OF THE FILM STARCHES: 1 – *D. SP.*, 2 – *D. PIPERIFOLIA*, 3 – *D. TRÍFIDA*, AND STARCH MIXTURES: 4, 5, 6, 7, 8, 9, 10; AND FROM THE NATIVE STARCHES: A – *D. SP.*, B – *D. PIPERIFOLIA*, C – *D. TRÍFIDA*, AND STARCH MIXTURES: D, E, F, G, H, I, J.

Film Samples	XRD Results	Starch Samples	XRD Results
	Degree of relative crystallinity (%)		Degree of relative crystallinity (%)
1	15.81 ± 0.57^{g}	**a**	13.50 ± 0.12^{h}
2	21.07 ± 0.31^{c}	**b**	22.02 ± 0.58^{b}
3	23.88 ± 0.25^{b}	**c**	23.98 ± 0.39^{a}
4	19.45 ± 0.42^{e}	**d**	16.60 ± 0.57^{g}
5	20.03 ± 0.29^{d}	**e**	17.54 ± 0.37^{f}
6	26.46 ± 0.55^{a}	**f**	20.75 ± 0.24^{c}
7	19.43 ± 0.36^{e}	**g**	18.46 ± 0.46^{e}
8	17.02 ± 0.59^{f}	**h**	14.72 ± 0.21^{h}
9	21.02 ± 0.63^{c}	**i**	18.31 ± 0.45^{e}
10	24.60 ± 0.12^{b}	**j**	19.12 ± 0.13^{d}
p (normality)*	0.06	p (normality)*	0.05
p (Levene)*	0.004	p (Levene)*	0.002
p (Anova)**	-	p (Anova)**	-
p (Kruskal Wallis)***	< 0.001	p (Kruskal Wallis)***	< 0.001

a) (*) Probability values obtained by Anderson-Darling test for normality and by the Levene test for homogeneity of variances. b) (**) Probability values obtained by one-way ANOVA. c) (***) Probability values obtained by Kruskal–Wallis test. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).

SOURCE: The author (2018).

The degree of relative crystallinity obtained for the film samples are attributed to a more ordered crystalline structure (changes in the orientation of the double helices within the crystals) due to retrogradationfollowing cooling and storage of the filmogenic solutions (JIMÉNEZ et al., 2012; LI et al., 2015; PANKAJ et al., 2015). The starch film samples 1 (100 % *D. sp.* native starch) and 8 (70/15/15 % of *D. sp.*/*D. piperifolia*/*D. trífida*), developed with the native starch species, *D. sp.*, that had higher apparent amylose content, showed decreased relative crystallinity of ≅ 15 and 17 %, respectively. Starch with high amylose content is expected to show higher crystallinity once amylose molecules recrystallize rapidly during cooling soon after gelatinization resulting in brittle films. The plasticizers are applied to the film-forming dispersions to decrease intermolecular forces (amylose and amylopectin attraction) interrupting hydrogen bonding along the polymer chains (ARVANITOYANNIS; BILIADERIS, 1998). Thus, the amount of glycerol used was compatible to the native *D. sp* starch matrix lowering the retrogradation process and consequently the crystallinity of the films 1 and 8. Plasticization occurs in the amorphous region which has higher molecular mobility, and the ability of plasticizers to interrupt hydrogen bonding along

the polymer chains depends both on the plasticizer type and the amount (ARVANITOYANNIS; BILIADERIS, 1998; BOURTOOM, 2008; JIMÉNEZ et al., 2012; CAZÓN et al., 2017). Moreover, when film crystallinity is lowered, tensile strength decreases and elongation at break increases (DONHOWE; FENNEMA, 1993; VELASQUEZ et al., 2015) as observed for the starch-based samples 1 and 8 (Table 4). The crystallinity of starch-based films depends on several factors including drying and storage conditions (temperature and relative humidity) as well as the content of plasticizer (JIMÉNEZ et al., 2012). Using diffractograms, it can be verified that the doublet existing in the starch samples a, b and c (mixed) were not visualized in the derived films and the intensity and area of the angles showed a slight increase. When a new angle is formed, as observed for film sample 2, it can be attributed to lack of intermolecular interactions between components of the films (CHEN; LIU; ZHANG, 2014).

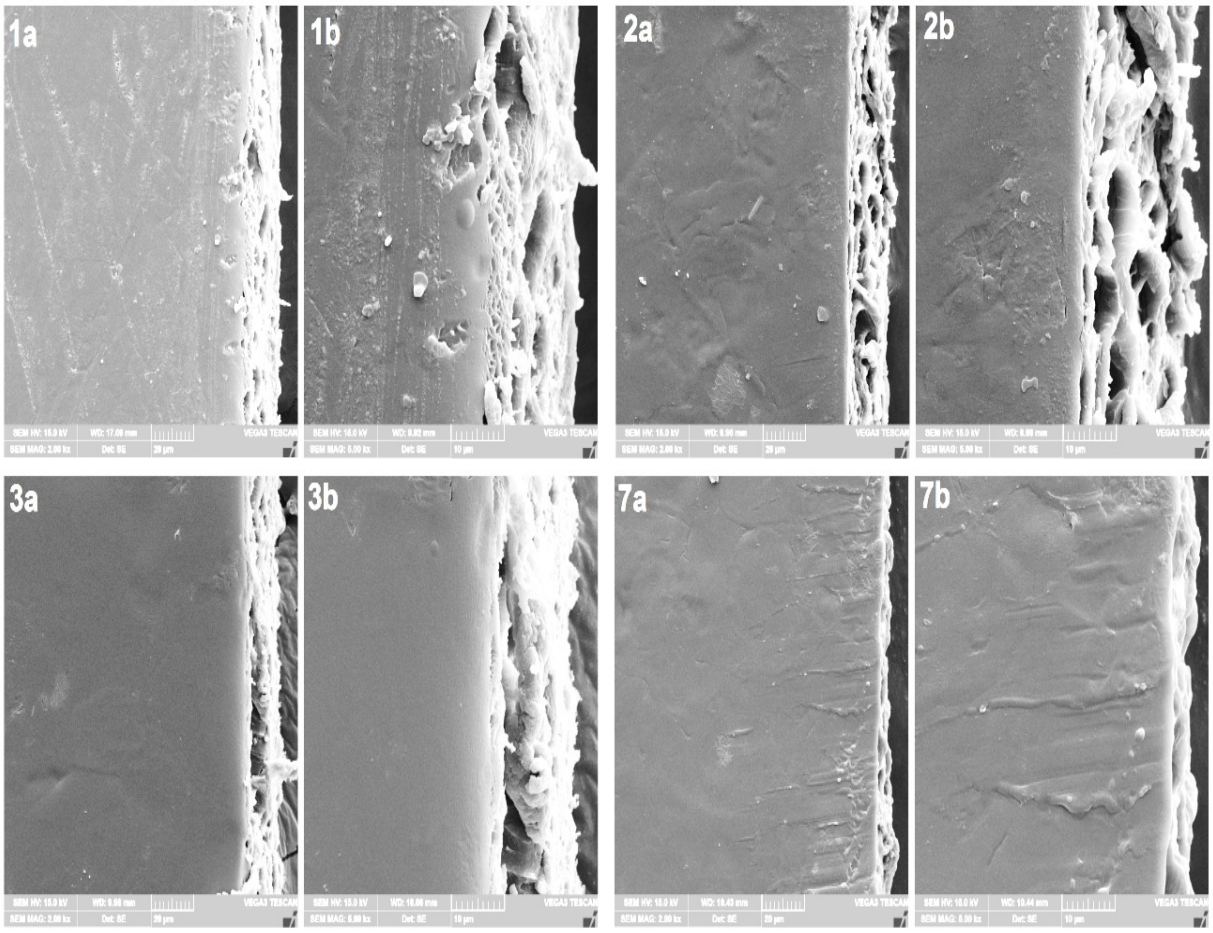
3.2.8.2 Scanning Electron Microscopy (SEM)

Scanning electron micrographs of the surfaces of the fractured native starch films (samples 1 to 3), and represeting the films from the starch mixtures, sample 7 (33/33/33 of *D. sp.*/*D. piperifolia*/*D. trífida*) are shown in Fig. 5 amplified 2000x (a) and 5000x (b).

For all film sample superficies, whithered ghost granules of starch were observed. However, sample 3 shows the most homogeneous and smoother surface. Despite ghost granules, the samples surfaces were a continous matrix without cracks or pinholes showing good structuralintegrity. The plasticizer increased the association between the long-chain polymers resulting in homogenous film matrices as observed for the samples studied, particularly sample 3 (100 % *D. trífida* native starch) which corroborated with the suggestion of its better interation with the amount of the glycerol applied. Plasticized films are expected to show smooth surfaces without pores or cracks, and a compact structure (MALI et al., 2002). Similar micrographs were obtained by Mali et al. (MALI et al., 2002) who developed films with different concentrations of *D. alata* yam starch and glycerol. Sample 3, the film from the *Dioscorea trífida* starch, developed in this study showed better characteristics including no cracks or pinholes, white strokes or lines than the film

developed by Gutiérrez et al. (GUTIÉRREZ et al., 2015b) that applied the same starch species modified by cross-linking with sodium trimetaphosphate.

FIGURE 5. SEM MICROGRAPHS OF THE NATIVE STARCH-BASED FILMS (1 – *D. SP*; 2 – *D. PIPERIFOLIA* AND 3 – *D. TRÍFIDA*), AND FROM NATIVE STARCH MIXTURE-BASED FILM 7 (33/33/33 % OF *D. SP*/*D. PIPERIFOLIA*/*D. TRÍFIDA*).



SOURCE: The author (2018).

3.2.9 Color

For the color determination, a CIE-lab scale was used to obtain the degree values of lightness (*L**) with values ranging from 0 (black) to 100 (white), redness (*+a**) or greenness (*-a**), and yellowness (*+b**) or blueness (*-b**) of the film samples (HORNUNG et al., 2015, 2016, 2017a, 2017c) and the results are shown in Table 5.

TABLE 6. COLOR VALUES OF THE FILMS FROM NATIVE STARCHES: 1 – *D. SP.*, 2 – *D. PIPERIFOLIA*, 3 – *D. TRÍFIDA*, AND STARCH MIXTURES: 4, 5, 6, 7, 8, 9, 10.

Samples	***Colour Results***		
	L*	***a****	***b****
1	24.50 ± 1.03^{b}	-0.12 ± 0.11^{h}	-0.55 ± 0.26^{i}
2	21.10 ± 1.00^{h}	1.30 ± 0.45^{a}	10.22 ± 1.13^{a}
3	22.57 ± 0.81^{e}	0.32 ± 0.12^{f}	0.36 ± 0.18^{j}
4	26.89 ± 0.70^{a}	0.20 ± 0.15^{g}	3.95 ± 0.38^{f}
5	21.66 ± 0.44^{f}	0.59 ± 0.05^{d}	0.86 ± 0.14^{h}
6	23.14 ± 0.60^{d}	0.94 ± 0.16^{b}	5.92 ± 0.51^{c}
7	21.36 ± 1.43^{g}	0.54 ± 0.12^{e}	4.96 ± 0.55^{d}
8	24.35 ± 1.77^{c}	-0.05 ± 0.10^{i}	2.41 ± 0.78^{g}
9	21.33 ± 0.75^{g}	0.21 ± 0.15^{g}	8.33 ± 0.83^{b}
10	19.30 ± 0.43^{i}	0.66 ± 0.13^{c}	4.42 ± 0.51^{e}
p (normality)*	0.55	0.86	0.88
p (Levene)*	0.001	< 0.0001	< 0.0001
p (Kruskal Wallis)***	< 0.001	< 0.001	< 0.001

a) (*) Probability values obtained by Anderson-Darling test for normality and by the Levene test for homogeneity of variances. b) (***)Probability values obtained by Kruskal–Wallis test. Means followed by the same letters in the same column do not differ statistically by Duncan test ($p < 0.05$).

SOURCE: The author (2018).

For all parameters analyzed, the films showed significant differences at p<0.05 and little tendency to lightness with sample 10 having the lowest value (19.30). Samples 1 (film from *D sp.* native starch) and 8 (film from mixture containing *D sp.* at the highest starch content) showed a smooth tendency to greennes (*-a** value of -0.12 and -0.05, respectively). The film from the *D. piperifolia* native starch (sample 2) had the highest value for redness (+ 1.30) among the samples. Regarding the degree of yellowness (*+b**) and blueness (*-b**) results, sample 2 showed the strongest tendency to yellow (+ 10.22) (Fig. 2.) and the films which contains it maintained the yellow tendency predominance (samples in crescent order: 9, 6, 7, 10 and 4). The

results shown by the samples for color corroborates with the ones obtained for light transmission that could indicate the application of these film as good light barriers. The *D. piperifolia* Humb. var. Wild and *D. trífida* tubers contain pigments such as carotenoids (RODRIGUES et al., 2012) and anthocyanins (RAMOS-ESCUDERO et al., 2010) that may be carried over to the starch during its extraction, which could explain the color properties of their starches and starch films.

4 Conclusions

In this work, biodegradable native starch and starch mixtures-based films were produced by casting solutions with novel *Dioscoreaceae* starch sources from Brazil. The present green development method of mixing yam native starches from the species *Dioscorea sp*., *Dioscorea piperifolia* and *Dioscorea trífida*, enhanced in over 100 % the biopolymer starch film mechanical characteristics. For tensile strength it can be highlidhted the results obtained by samples 6 (22 MPa), 9 (19 MPa) and 10 (20 MPa) and for elongation at break by samples 4 (33 %) and 8 (39 %). Moreover, all films presented microbiological and light deterioration preventing properties for food products by their lower moisture content (≅ 11%), and light transmission rate (ranging from 12 to 16 %) as confirmed by the color values, even though their transparency was increased. Although the mixing process did not change significantly the WVP properties of the Dioscoreaceae native starch films, it is important to measure this parameter to guide the application of the films for different products. The solubility of the film developed of native *D. sp.* starch was improved in 8 % when mixed with the *D. piperifolia* and the *D. trífida* starches, representing a better biodegradability characteristic. Although the melting temperatures increased in a range of 10 to 40 ºC and the glass transition temperatures were decreased from 52 to 41 ºC they are proper to maintain the film integrity under room temperatures. XRD patterns and SEM micrographs revealed that the *Dioscorea* starch films maintained the “C-type” molecular characteristic and that the amount of glycerol applied was compatible with the starches generating smooth and compact film matrices, respectively. The findings suggest that the films produced may provide different characteristics for use in numerous applications depending on the desired properties.

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Conflict of interest statement

The authors, Polyanna Silveira Hornung, Suelen Ávila, Kabo Masisi, Lovemore Nkhata Malunga, Marcelo Lazzarotto, Egon Schnitzler, Rosemary Hoffmann Ribani and Trust Beta, state that there is no conflict of interest.

Novelty of the work

-For the first time, films were developed from native starch mixtures;
-From an environmental-friendly process, strong and flexible films were developed;
-Native starch films' mechanical characteristics were improved over 200% for elongations at break.

CHAPTER 5

NATURAL BIOACTIVE STARCH FILM FROM AMAZON TURMERIC (*CURCUMA LONGA* L.)

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Natural bioactive starch film from Amazon turmeric (*Curcuma longa* L.)

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Abstract

It is evermore imperative to develop green processes and products due to the environmental and health problems facing modern society. Thus, the aim of this study was as follows: to use HPLC-MS to identify and quantify the phenolic compounds in Amazon *Curcuma longa* L. starch; to develop a bioactive starch film and to characterise its phenolic compounds (HPLC-MS) and antioxidant capacity (DPPH, ABTS, and ORAC), as well its thickness and granular structure (SEM), mechanical (tensile strength and elongation at break) and colour (CIE-lab scale: *L**, *a**, *b**) properties. HPLC-MS revealed eight major phenolic compounds in the *C. longa* starch and six in its film. The starch and the film from *C. longa* had high levels of antioxidant capacity, 65 – 92% (as measured by DPPH and ABTS). Furthermore, *C. longa* exhibited a smooth structural surface and strong resistance to tensile force, as well as maintaining its elasticity as measured by mechanical assays (tensile strength and elongation at break). Based on the obtained results a novel biodegradable starch film was obtained applying a single matrix, the Amazon *Curcuma longa* L. starch.

Keywords: HPLC-MS; native starch; green process; biodegradable packing.

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1 Introduction

There is a growing need to develop packaging materials, such as films and coatings, from renewable matrices or industrial wastes (GUTIÉRREZ; ALVAREZ, 2017; MANIGLIA et al., 2014). This is driven by the surge in productive economic processes and the need to preserve the environment and to consume less processed foods. Biodegradable starch packaging, which can also act as antioxidants and antimicrobials, serves these interests by lowering the levels of additives incorporated in foods to preserve them (SALGADO et al., 2015; TALÓN et al., 2017). Starch is a well-known, renewable raw material that presents significant technological features including gelling, whitening, thickening and emulsifying. These properties makes it one of the most used base materials and the most investigated in terms of the development of biodegradable films (GUTIÉRREZ; ALVAREZ, 2017; HORNUNG et al., 2017; LI et al., 2015; PIÑEROS-HERNANDEZ et al., 2017; SHAH et al., 2016).

With established applications in a variety of industrial fields (food, pharmaceuticals, textiles, paper and chemicals), starch is mainly composed of two glucose polymers, one of which is linear (amylose) and the other is branched (amylopectin), which are linked by α-1,4 and α-1,6 connections at branched points. It is the amylose molecule that is responsible for the formation of vigorous and flexible films (JIMÉNEZ et al., 2012; MALI et al., 2002). Amylose has a molecular weight of $\cong 10^5$ - 10^6 and it composes the amorphous and the single helical conformation of starch granules (LI et al., 2015). However, amylopectin has a molecular weight of $\cong$ 10^7 - 10^9 and its branches form clusters of double helices that aggregate into crystallites in starch granules (LI et al., 2015). Due to factors such as their botanical source, growing conditions, granule size and shape, and amylose and amylopectin ratio, native starches have different properties. Therefore, it is essential to study and

investigate non-conventional starch sources (HORNUNG et al., 2017; JIMÉNEZ et al., 2012).

Curcuma longa L. belongs to the Zingiberaceae family genus and it has been extensively investigated as a medicinal and pigment source. Popularly known as turmeric, this rhizome originates in South Asia and has more than 100 catalogued species (VAN HUNG; VO, 2017). The Curcuma rhizome is widely cultivated and easily obtained: India is the main producer and consumer (ANUBALA; SEKAR; NAGAIAH, 2014). Brazil has an impressive cultivation of turmeric in the centre-west that corresponds to 26% of the production of the state of Goiás, demonstrating the potential to supply the demands of the internal market (BERNI et al., 2014). Moreover, its cultivation stimulates the economic growth of rural families in the northeast region of Brazil (BERNI et al., 2014). The main use of the powdered rhizome of Curcuma is to extract oleoresin for pigments known as curcuminoids (curcumin; desmethoxycurcumin and bisdemethoxycurcumin) (BERNI et al., 2014; MANIGLIA et al., 2014; MUSSO; SALGADO; MAURI, 2016). India has 78% of the global turmeric market and pharmaceutical applications account for over 52%. The quest for natural colourants in food and beverages has increased the demand for *Curcuma longa* L. (GLOBAL MARKET INSIGHTS, 2016). According to Van Hung and Vo (VAN HUNG; VO, 2017), the pigment only accounts for 2-8% of the powdered rhizome: its starch content is a residue that represents 78-85% of the initial matter and it has no commercial value (NGUYEN et al., 2014). *C. longa* rhizome starch represents an attractive low-cost renewable base material for bioplastics (NGUYEN et al., 2014). Apart from its important phenolic compounds, which impart antioxidant, antibacterial and anticancer properties, another advantage of *C. longa* rhizome starch in relation to developing starch films is its apparent amylose content of 48% (ANUBALA; SEKAR; NAGAIAH, 2014; VAN HUNG; VO, 2017).

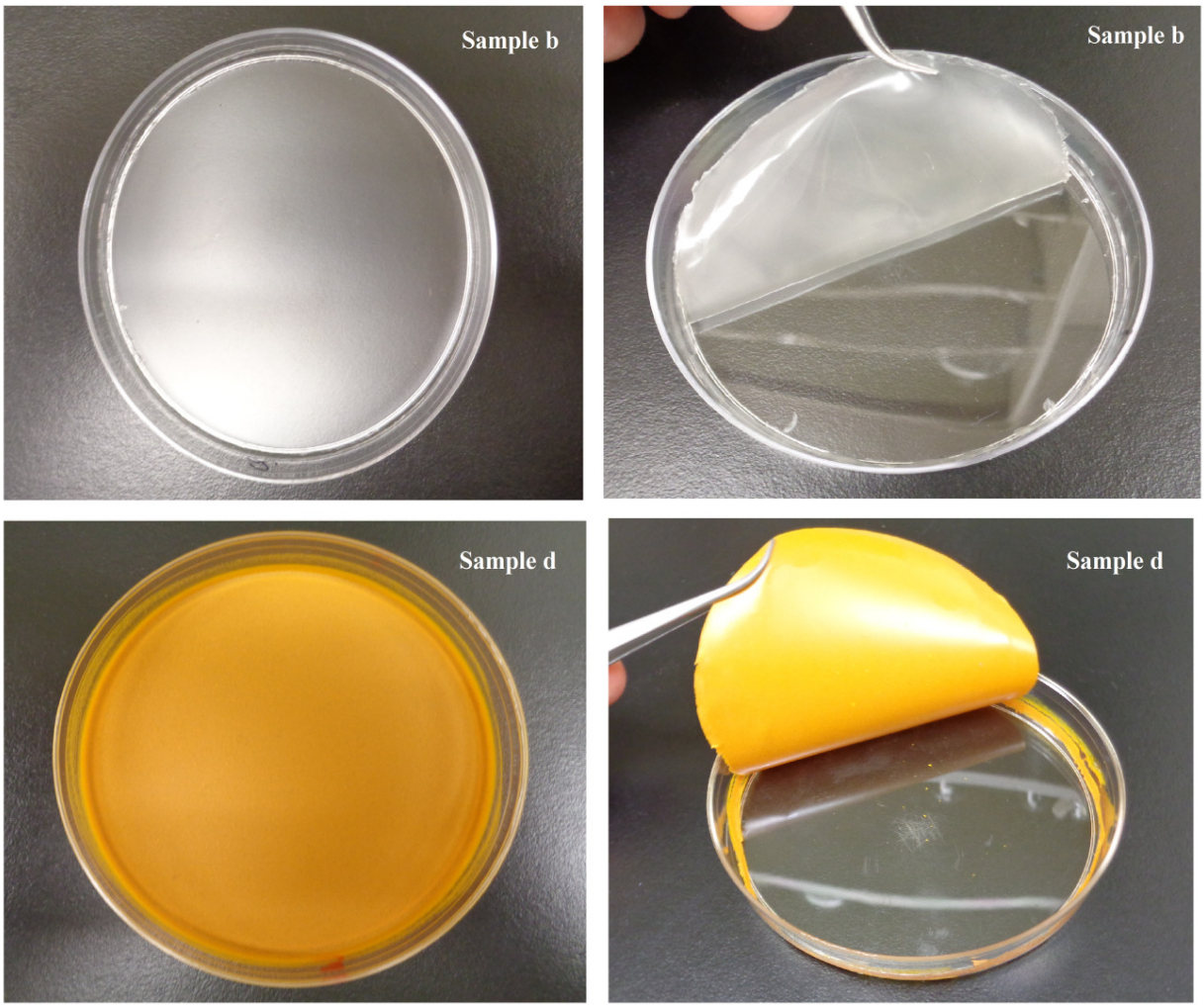
Considering that starch, which is a major component in turmeric rhizomes, has been poorly investigated regarding its application as an active film, this study focused on characterising the phenolic compounds of Amazon turmeric starch (*Curcuma longa* L.), its antioxidant capacity (DPPH, ABTS, ORAC), and its application as a naturally active starch film. The film was also investigated for its structural and mechanical properties.

2 Materials and Methods

2.1 Samples

The native turmeric rhizomes (*Curcuma longa* L.) were harvested in the crop area of Aripuanã city, Mato Grosso, in the Amazon region of Brazil (10º10'00" S / 59º27'34" W). The starch was extracted as described in a previous study (HORNUNG et al., 2017). The starch film was prepared according to the procedure described by Pauli et al. (PAULI et al., 2011). Commercial corn starch was used as a control sample. The samples were coded as follows: a – corn starch; b – corn starch film; c – native turmeric starch; and d – turmeric starch film (Fig. 1).

FIGURE 1. IMAGES OF SAMPLE B – CORN STARCH CONTROL FILM; SAMPLE D – *CURCUMA LONGA* STARCH FILM.



SOURCE: The author (2018).

2.2 Chemicals and solvents

HPLC grade methanol, hexane, and ethyl acetate were obtained from Fisher Scientific Company (Ottawa, ON, Canada). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH); (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); fluorescein and the phenolic compound standards (4-hydroxybenzaldehyde; vanillin; *p*-coumaric acid and ferulic acid) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

2.3 Characterization Methods

The methods, items 2.3.1 to 2.3.5 and 2.4 were performed for all samples (Corn starch and film; Turmeric starch and film). The analyses described in item 2.3.5 to 2.3.8 were realized only for the corn and turmeric starch films.

2.3.1 Physicochemical analyses

The total starch content of the turmeric sample studied was quantified following the methodology described by Jiang et al. (JIANG et al., 2010). The moisture content was determined by desiccation at 105 ºC (oven); total proteins by the Kjeldahl method; total lipids by exhaustive extraction with petroleum ether in Soxhlet apparatus; and fixed mineral residue by incineration in a muffle at 550 ºC using the AOAC ((2000), 2000) protocols 930.15, 990.03, 920.39 and 942.05, respectively. The total carbohydrate content was obtained by difference to 100%.

2.3.2 Extraction of free and bound phenolic fractions

The turmeric and corn starch films were lyophilised and ground to obtain a powder. Extracts of free and bound phenolic fractions from the starch films powder and of the turmeric and corn starch were obtained according to the methodology described by the authors (ZHANG et al., 2015) with some modifications. For the free fraction, 100 mg of each sample (in triplicate) was extracted twice with 80% acidified methanol (1% HCl) and sonicated for one hour at room temperature prior to

centrifugation at 1000 rpm for 10 minutes. The supernatants were combined and dried under nitrogen at 38 ºC and then reconstituted with 300 µL of 50% methanol. For the bound fraction, 50 mg of each sample (in triplicate) was weighed in 5 mL glass tubes. Then, 2 mL of 4 M NaOH was added and the mixture was left in the refrigerator overnight. The samples were then acidified to pH 1.0 with 6 M HCl (≅ 1 mL), vortexed and centrifuged at 10000 rpm at 4 ºC. The supernatant was collected and the remaining sample was washed with 500 µL of pure H_2O, vortexed and centrifuged. The supernatants were pooled together and extracted with 2 mL ethyl acetate. The ethyl acetate fraction was dried under nitrogen at 35 ºC and reconstituted with 300 µL of 50% aqueous methanol. After the reconstitution, the samples were filtered through a syringe filter (0.22 µm, HV Millipore, DuraPore). The extracts were stored at -20 ºC until the moment of analysis.

2.3.3 Total phenolic content (TPC)

The TPC was obtained using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (SINGLENTON; ROSSI, 1965), with some modifications. An appropriately diluted sample (20 µL) was added to 150 µL of 10-fold freshly diluted Folin-Ciocalteu reagent in a microplate. Then 150 µL of sodium carbonate solution (75 g/L) was added and the samples were kept in the dark for one hour at room temperature. The absorbance was measured at 725 nm in an Epoch Microplate spectrophotometer (Synergy-BIOTEK, USA): ferulic acid was used as the standard. The results were expressed as µM of ferulic acid equivalents (FAE) per milligram of the samples (dry weight basis).

2.3.4 Antioxidant capacity assays

The DPPH radical scavenging activity and ABTS radical scavenging activity were performed in 96-well microplates and read (6 x for each sample) in an Epoch Microplate spectrophotometer (Synergy-BIOTEK, EUA).

2.3.4.1 DPPH radical scavenging activity

The DPPH assay was carried out according to the method of Brand-Williams et al. (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). Briefly, 10 µL of the sample extracts or standards were added to 190 µL of DPPH radical solution (60 µmol/L), which was freshly made in methanol. After 30 minutes incubation in the dark at room temperature the absorbance was obtained at 515 nm. The $DPPH^{\bullet}$ radical scavenging activity was obtained as % DPPH = [(DPPH absorbance - sample absorbance)/DPPH absorbance]*100. The DPPH radical scavenging activity of the sample extracts was expressed as µM of Trolox equivalents (TE) per milligram of the sample (dry weight basis) using a standard curve of Trolox ranging from 100 to 700 µM.

2.3.4.2 ABTS radical scavenging activity

For the ABTS assay, the methodology described by Re et al. (RE et al., 1999) was followed, with minor modifications. Stock solutions of 7 mmol/L ABTS solution and 2.45 mmol/L of potassium persulfate solution were prepared. The working solution was mixed with 3 mL of each stock solution and allowed to react for 16 h at room temperature in the dark. The solution was then diluted by mixing 4.0 to 4.5 mL of ABTS radical cation solution with 250 mL of distilled water to obtain an absorbance of 0.70 at 725 nm. The $ABTS^{\bullet+}$ radical scavenging activity was calculated as % discolourisation = [(ABTS absorbance - sample absorbance)/ABTS absorbance]*100. The results were compared with a Trolox standard curve and expressed in µM of Trolox equivalents (TE) per milligram of the sample (dry weight basis).

2.3.4.3 Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed to quantify the peroxyl radical-scavenging activity of the samples; it was based on the procedure described by Qiu et al. (QIU; LIU; BETA, 2010). The solutions were automatically transferred plate to plate with a Precision 2000 automated microplate pipetting system (BIO-TEK Instruments, Inc., Winooski, VT). Subsequently, a FLx 800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used to obtain an excitation wavelength of

485/20 nm and an emission wavelength of 528/20 nm; it was programmed to record the fluorescence every minute for 50 min after the addition of AAPH. The curve of the fluorescence decay was integrated using KC4 3.0 software. Each sample was measured three times and the results were expressed as µM of Trolox equivalents (TE) per milligram of the sample (dry weight basis).

2.3.5 HPLC–DAD–Q-TOF–MS analysis of phenolic compounds

The HPLC-MS analysis of phenolic compounds was conducted using an HPLC (Waters 2695) machine equipped with a photodiode array detector (PDA) (Waters 996) and autosampler (717 plus, Waters), coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF MS), as described by Chen et al. (CHEN et al., 2016), with minor modifications. The analytical column was a 150mm x 4.6 mm, Gemini 5 µm C18 110A column (Phenomenex, Torrance, California, USA). The mobile phase consisted of: A (0.1% acetic acid in water) and B (0.1% acetic acid in methanol). The HPLC separation conditions were 35 ºC column temperature, 1.0 mL/min flow rate, and 10 µL injection volume. A 70-minutes gradient was used as follows: 0–11 min, 9–14% B; 11–14 min,14–15% B; 14–17 min, 15% B; 17–24 min, 15–16.5% B; 24–28 min,16.5–19% B; 28–30 min, 19–25% B; 30–36 min, 25–26% B; 36–38 min, 26–28% B; 38–41 min, 28–35% B; 41–46 min, 35–40% B; 46–48 min, 40–48% B; 48–53 min, 48–53% B; 53–65 min, 53–70% B; 65–66 min, 70–9% B; 66–70 min, 9% B. The phenolic compounds were detected at a wavelength of 280 nm and 320 nm. The identification was performed by comparing the retention time and spectral matching with external standards, which was confirmed by Q-TOF–MS. The Q-TOF–MS was calibrated using sodium iodide for the negative mode through the mass range of 100–1000. A resolution of 5000 was achieved. Full mass spectra were recorded in negative mode by using a capillary voltage of 900 kV and a cone voltage of 15 V. The flow rates of the desolvation gas (N_2) and cone gas (He) were 900 and 50 L/h, respectively. The desolvation gas temperature and the ion source temperature were set at 250 and 120 ºC, respectively. The MS/MS spectra were acquired by using a collision energy of 33 V.

2.3.6 Thickness and Microscopy

The film thickness was determined at 15 random positions with a digital micrometer, using a sensitivity of 0.001 mm (Mitutoyo – Absolute DIGIMATIC, Japan).

The surface of the starch film samples were observed using a scanning electron microscope (SEM) (Tescan, VEGA 3, Kohoutovice, Czech Republic) under an acceleration voltage of 15 kV, a reading scale of 20 µm for magnification of 2000x and 10 µm for magnification of 5000x after the samples had been coated with plasma of gold and palladium

2.3.7 Glass transition

The glass transition temperature (T_g) of the films were determined using a differential scanning calorimeter (DSC Q200, TA Instruments) (LUCHESE et al., 2015).

2.3.8 Tensile strength (TS) and elongation at break (EB)

The mechanical properties were determined according to the D882-91 method (ASTM, 1996). The parameters of tensile strength (TS - MPa) and elongation at break (EB - %) were analysed in a TA.XT Plus texture analyser (Texture Technologies Corp., Hamilton, Canada). The method previously described by (GALDEANO et al., 2013) was used, with some modifications. The films (8.2 x 8.2 cm) were fixed in the rig probe (TA-108N, Large Film Extensibility) and a 1/2" dial ball cylindrical probe (TA-18) was used to penetrate the films at a speed of 0.8 mm/s. Ten samples were tested in this analysis.

2.3.9 Colour analysis

The colour determinations were performed with a Konica Minolta colorimeter (model CM-3500d, Minolta, CO., Japan) using the 0.3 cm aperture port, dual 18-element silicon photodiode array, and a wedge-shaped continuous interference filter

to record the CIELAB space. The method was followed as described in the literature (ASTM, 2005; ROBERTSON, 1977).

2.4 Statistical analysis

The results were presented as mean ± standard deviation (SD) of triplicated determinations. The data were analysed by ANOVA using SAS statistical software (version 9.3, SAS Institute Inc., Cary, NC, USA). Duncan's multiple range tests were used to evaluate the significant difference between the means. The significance level was defined at $p < 0.05$.

3 Results and Discussion

The proximal composition presented by the turmeric starch sample studied was as follows: lipids 0.47 %; proteins 1.70 %; ashes 2.17 %; moisture 16.45 %; starch content 57.98 %; total carbohydrates content 21.23 %.

Similar result for total starch content (56 %) was found by literature (KUTTIGOUNDER; LINGAMALLU; BHATTACHARYA, 2011) that isolated the starch from commercial turmeric powder (Karnataka, India), and lower values, ranging from 45.24 to 48.48 %, were reported by Sajitha and Sasikumar (SAJITHA; SASIKUMAR, 2015) that studied four different species of turmeric.

3.1 Total phenolic content (TPC)

Phenolic compounds are widely distributed in nature; more than 8,000 have been identified and the list keeps growing. They have been extensively studied due to their potential uses in relation to human health (in pharmaceuticals and food processing), as antioxidants, and as a chronic disease-preventing agent (CHEN et al., 2016; CHEYNIER, 2012; LORDÊLO et al., 2010). The free and bound phenolic fractions of the control samples (a – commercial corn starch, b – commercial corn starch film) and of the *C. longa* native starch and its derivate film (samples c and d, respectively) were investigated and the results are presented in Table 1.

TABLE 1. TOTAL PHENOLIC CONTENT (TPC) AND ANTIOXIDANT CAPACITY BY DPPH, ABTS AND ORAC ASSAYS OF: (C) TURMERIC STARCH AND (D) TURMERIC STARCH FILM.

Samples	TPC µM FAE /mg			ORAC µM TE /mg			DPPH µM TE /mg			ABTS µM TE /mg		
	Free	Bound	Total	Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
c	67.6 ± 7.1[a]	119.1 ± 36.5[a]	187.0 ± 43.6[a]	10.1 ± 2.4[a]	58.7 ± 18.1[a]	68.8 ± 20.5[a]	71.4 ± 17.0[a]	313.1 ± 46.5[a]	384.5 ± 63.6[a]	267.0 ± 20.7[a]	421.3 ± 0.5[a]	688.0 ± 21.2[a]
d	23.0 ± 3.9[b]	89.9 ± 18.0[b]	113.0 ± 22.0[b]	5.1 ± 1.7[b]	52.1 ± 12.1[b]	57.2 ± 13.8[b]	34.1 ± 2.8[b]	248.0 ± 12.3[b]	281.8 ± 15.1[b]	263.0 ± 27.0[b]	349.0 ± 35.2[b]	612.0 ± 62.2[b]

Samples	DPPH$^{\cdot}$ scavenging activity % /mg			ABTS$^{\cdot+}$ scavenging activity % /mg		
	Free	Bound	Total	Free	Bound	Total
c	12.8 ± 0.8[b]	57.1 ± 1.52[a]	70.0 ± 2.3[a]	78.8 ± 6.2[a]	92.5 ± 0.1[a]	100.0
d	18.2 ± 1.28[a]	47.5 ± 8.6[b]	65.8 ± 9.9[b]	60.4 ± 2.12[b]	82.6 ± 15.7[b]	100.0

Values in each column with different letters are significantly different ($p < 0.05$).
SOURCE: The author (2018).

Phenolics were not detected for the control samples, corn starch (a) and corn starch film (b). The turmeric starch (c) had 187 µM FAE/mg, and the turmeric starch film (d) also presented a high total phenolic content (113 µM FAE/mg) even after being held for three minutes at 90 ºC for gelatinisation, thereby demonstrating stability to heat. In contrast to a recent study by (VAN HUNG; VO, 2017) regarding starches from two varieties of turmeric rhizomes (*Curcuma longa* and *Curcuma caesia* from Lam Dong, Vietnam), the Amazon *C. longa* turmeric starch (sample c) showed TPC concentration ≅71 x higher (961.26 mg /g starch) than the Vietnamese *C. longa* turmeric starch (13.4 mg /g starch). The differences between the two aforementioned cultivars are likely to have been due to the growing conditions, climate and harvesting method, as well as the procedures and/or reagents used in the analysis. The evaluation of phenolic content and antioxidant activity are important parameters that can help to establish quality parameters and market price (ANUBALA; SEKAR; NAGAIAH, 2014).

The bound phenolics showed higher potential physiological functions, including antioxidant capacity and bioavailability (ACOSTA-ESTRADA; GUTIÉRREZ-URIBE; SERNA-SALDÍVAR, 2014). Bound phenolics are covalently bound to carbohydrates and the cellular wall structural components of plants (CHEN et al., 2016). Table 1 shows that the highest amount of bound phenolics was found for sample d and its relationship to the free phenolic fraction could be attributed to the

protecting action of the starch granule structure during gel formation for the starch film.

3.2 Antioxidant capacity

In the present study, ORAC, DPPH and ABTS assays were performed to evaluate the antioxidant capacity activity of the sample extracts (Table 1). DPPH and ABTS assays are based on the colour reducing properties of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, respectively, by electron transfer, while ORAC is based on hydrogen atom transfer (CHEN et al., 2016; RAGAEE; ABDEL-AAL; NOAMAN, 2006). No antioxidant activity was detected in the control samples (a and b) in all of the assays that were performed.

The bound fractions of samples c and d showed stronger antioxidant activity in all the antioxidant tests. The *C. longa* starch and its starch film phenolic content presented the highest antioxidant capacity by transferring electrons ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) rather than H atoms (ORAC). Nonetheless, the antioxidant capacity of the *C. longa* starch was only slightly affected by heat treatment, as previously shown by (SELVAM et al., 1995) regarding turmeric root (*C. longa* - India) extracts. Generally, films that present significant levels of phenolic compounds, as well as antioxidant capacity, are those to which commercial antioxidants (natural or synthetic), plant extracts and essential oils (among others) have been added following the gelatinisation of the starch or the heating of the polymeric solution (ARABESTANI et al., 2016; ARAÚJO et al., 2015; CIANNAMEA; STEFANI; RUSECKAITE, 2016; JIMÉNEZ et al., 2013; LÓPEZ et al., 2017; PIÑEROS-HERNANDEZ et al., 2017; SALGADO et al., 2015; SHAH et al., 2016; TALÓN et al., 2017).

The antioxidant capacity (DPPH expressed as % radical scavenging/mg of samples) (Table 1) of the total phenolics (free plus bound fractions) obtained for sample c – *C. longa* native starch, was 19% higher than reported for the same cultivar in a recent study (VAN HUNG; VO, 2017) and it only reduced by 6.0% after gelatinisation (sample d). As stated earlier, differences can be attributed to the climate and cultivation of the samples, as well as the reagents and methods used. For sample d (*C. longa* starch film), the DPPH radical scavending was 15% higher than the film developed with turmeric (*C. longa L.*) dye solvent extraction residue (MANIGLIA et al., 2014) and ≅ 97 % (when DPPH results were converted to µM of

TE/mg of film) higher than cassava starch-based film with different added concentrations of commercial ethanolic propolis extract (ARAÚJO et al., 2015). Moreover, a recent study (BITENCOURT et al., 2014) obtained a maximum of 55% ABTS antioxidant activity for gelatin-based films with added Curcuma ethanol extract (200 g CEE/100 of the film). The bound fraction of the film obtained in the present study (sample d), which was solely from native *C. longa* starch, reached 92.5% and surpassed the gelatin-based film. Using a single matrix to produce bioactive films such as *C. longa* starch represents an economic use of time and resources because the process is simplified. The total phenolic content analysis showed a high correlation with antioxidant activities (ORAC; DPPH; ABTS; r = 1), which was principally related to the bound phenolic fraction for both starch (sample c) and film (sample d).

3.3 HPLC–MS analysis of phenolic compounds

The free and bound extracts were subjected to HPLC-MS: four of the eight major phenolic compounds/peaks were successfully identified and quantified for samples c (*Curcuma longa* L. native starch) and d (*Curcuma longa* L. starch film). Based on their retention times, absorbance spectra and MS data, which are presented in Table 2 and Fig. 2 (supplementary data can be observed in the MS spectra of compounds 2 to 8), the compounds were identified and their concentration was obtained. The remaining four compounds (peaks 5 to 8) were characterised and expressed as ferulic acid equivalents. No phenolic compounds were detected in samples a (corn starch) and b (corn starch film), which was in agreement with the TPC results.

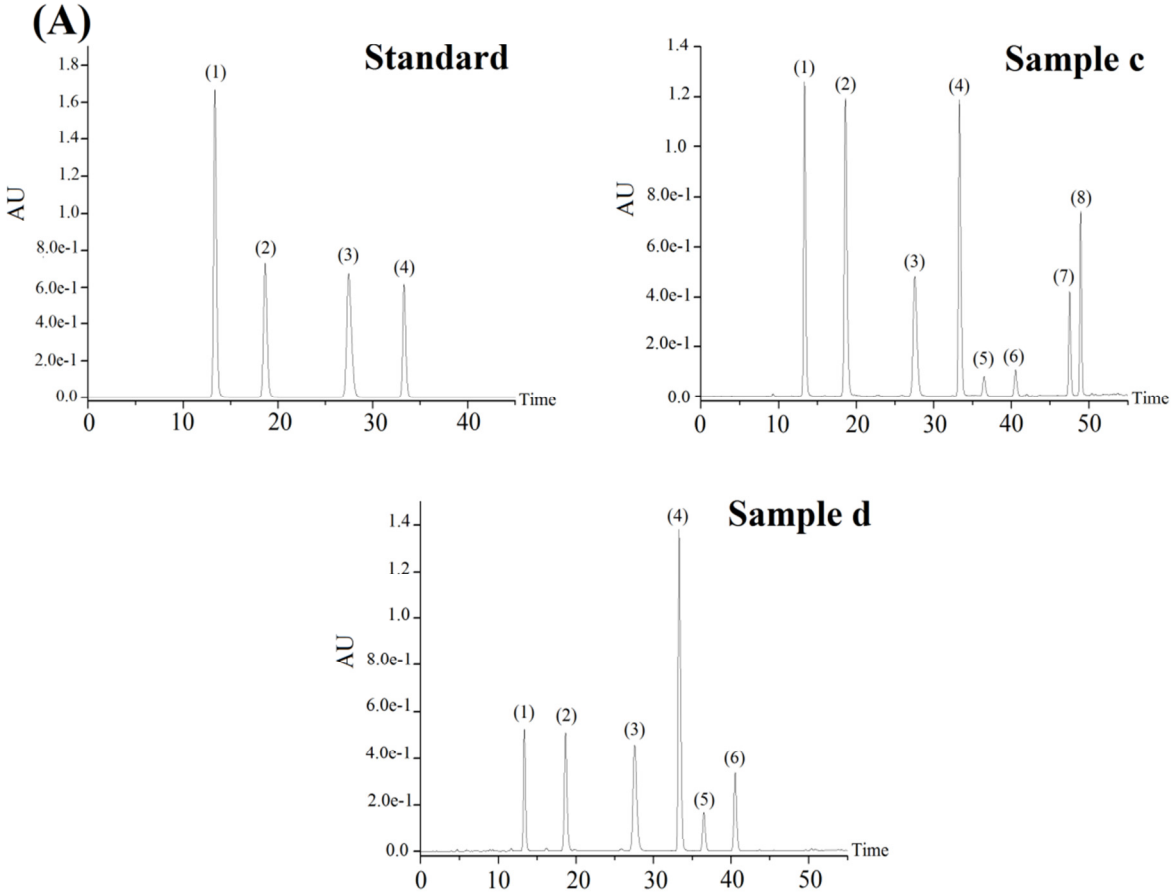
TABLE 2. CHARACTERIZATION OF PHENOLIC COMPOUNDS AND THEIR CONTENT IN THE FREE AND BOUND FRACTIONS OF (A) CORN STARCH; (B) CORN STARCH FILM; (C) TURMERIC STARCH AND (D) TURMERIC STARCH FILM.

Phenolic compounds	RT	Max. Abs.	[M-H-]	MS/MS (*m/z*)	Sample	Phenolic compound content (µg /g DW)		
						Free	Bound	Total
4-Hydroxybenz-aldehyde (peak 1)	13.16 ± 0.01	284	121	92	**a**	ND	ND	-
					b	ND	ND	-
					c	43.6 ± 2.9[a]	2,134 ± 64.1[a]	2,178 ± 67.0[a]
					d	23.7 ± 0.2[b]	890.4 ± 28.9[b]	914 ± 29.1[b]
Vanillin (peak 2)	17.97 ± 0.01	279	151	163, 108, 92	**a**	ND	ND	-
					b	ND	ND	-
					c	49.8 ± 3.8[a]	4,876 ± 236.8[a]	4,926 ± 240.6[a]
					d	19.0 ± 0.4[b]	2,024 ± 54.2[b]	2,043 ±54.6[b]
p-Coumaric acid (peak 3)	27.51 ± 0.08	309	163	119, 93	**a**	ND	ND	-
					b	ND	ND	-
					c	141.7 ± 8.8[a]	2,078 ± 96.7[a]	2,220 ± 105.5[a]
					d	92.4 ± 1.7[b]	2,055 ± 14.3[b]	2,147 ± 16.0[b]
Ferulic acid (peak 4)	32.93 ± 0.05	322	193	133, 89, 59	**a**	ND	ND	-
					b	ND	ND	-
					c	101.6 ± 4.4[a]	6,695 ± 63.2[a]	6,797 ± 67.6[a]
					d	68.3 ± 2.2[b]	5,446 ± 207.0[b]	5,514 ± 209.2[b]
Unknown (peak 5)	35.85 ± 0.02	323	161	117, 93	**c**	28.3 ± 0.9[a]	1,639 ± 10.1[a]	1,667 ± 11.0[a]
					d	22.8 ± 0.3[b]	745.6 ± 44.0[b]	768 ± 44.3[b]
Unknown (Peak 6)	39.59 ± 0.04	338	191	176, 105, 77	**c**	26.9 ± 1.1[a]	3025 ± 44.9[a]	3,052 ± 46.0[a]
					d	19.5 ± 0.1[b]	917.1 ± 49.8[b]	937 ± 50.0[b]
Unknown (peak 7)	47.40 ± 0.01	310	171	117	**c**	ND	1,165 ± 132.9	1,165 ± 132.9
					d	ND	ND	-
Unknown (peak 8)	48.65 ± 0.01	325	207	133, 115, 77	**c**	ND	2,603 ± 260.0	2,603 ± 260.0
					d	ND	ND	-

RT – retention time; Max. Abs. – Maximum absorbance; ND – not detectable; DW – dry weight.
Values in each column with different letters are significantly different ($p < 0.05$).

SOURCE: The author (2018).

FIGURE 2. (A) - HPLC CHROMATOGRAMS AT 280 NM, PEAKS: (1) 4-HYDROXYBENZALDEHYDE; (2) VANILLIN; (3) *P*-COUMARIC ACID; (4) FERULIC ACID; (5) TO (8) UNIDENTIFIED; (B) - MASS SPECTRA (*M/Z;* 4-HYDROXYBENZALDEHYDE). SAMPLE C –*CURCUMA LONGA* NATIVE STARCH; SAMPLE D – *CURCUMA LONGA* STARCH FILM.



SOURCE: The author (2018).

The corresponding chromatograms (Fig. 2) are shown at 280 nm to better visualise compounds (1) and (2), 4-hydroxybenzaldehyde and vanillin, respectively. Although they presented absorbance units (AU), slightly higher than compound (4) – ferulic acid (sample c), Table 2 confirms that ferulic acid was the predominant compound identified in the bound fractions of sample c (6,695 µg /g starch) and sample d (5,446 µg /g film). Contrarywise, *p*-coumaric acid (141.7 µg /g starch; 92.4 µg /g film) was predominant for the free fraction compound (3), followed by compound (4) (101.6 µg /g starch; 68.3 µg /g film). In contrast with the present study, a recent study (VAN HUNG; VO, 2017) focused on determining the curcumin content of the ethanolic extracts from Vietnamese *C. longa* and *C. caesia* starches using HPLC: the aforementioned study found curcumin to be the major phenolic compound, with values of 9600 µg/g, and 15 µg/g, respectively. No other phenolic

compound was reported to have been identified in that study. In comparison to the starches studied in the literature, the Amazon *C. longa* native starch (sample c) exhibited different phenolic compounds, which persisted even after the heat treatment to obtain its derivative film (sample d) and showed a different antioxidant mode of action, as presented by the antioxidant assays performed.

3.4 Thickness and SEM analysis

TABLE 3. THICKNESS, DSC, MECHANICAL PROPERTIES AND COLOR VALUES OF: (B) CORN STARCH FILM AND (D) TURMERIC STARCH FILM.

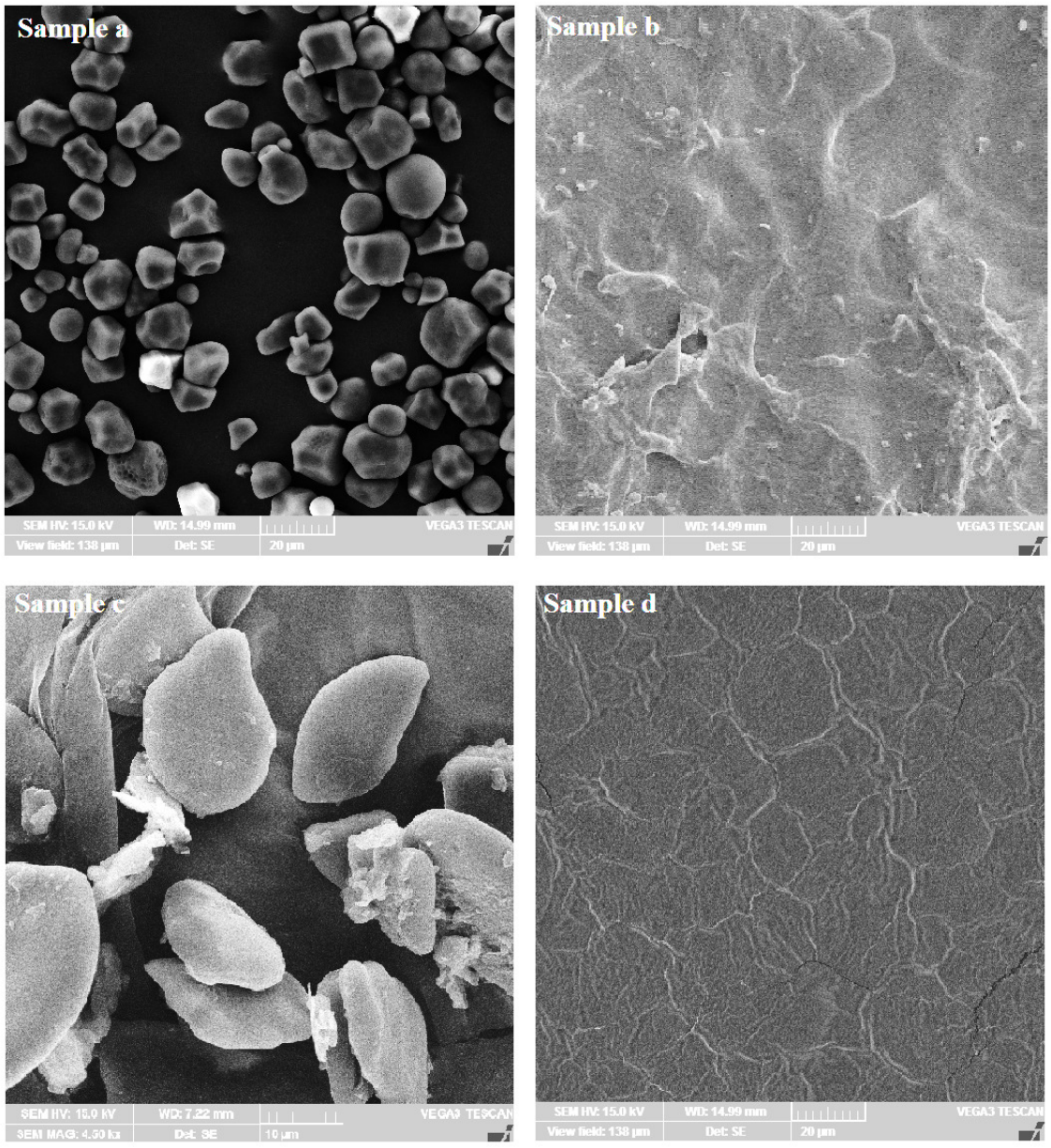
Samples	Thickness	SEM Result	DSC Result	Mechanical Properties			Colour				
	mm	*SG diameter*	T_g / ºC	TS MPa	EB (%)	*E* MPa	*L**	*a**	*b**	ΔE^*_{ab}	*WI*
b	0.15 ± 0.01[b]	12.1 ± 3.8	48.6 ± 0.9[a]	62.7 ± 7.1[b]	22.0 ± 0.5[a]	25.3 ± 2.5[b]	27.8 ± 1.7[b]	-0.5 ± 0.1[b]	-0.3 ± 0.3[b]	1.41 ± 0.02[b]	26.0 ± 0.9[b]
d	0.20 ± 0.01[a]	30.0 ± 2.0	48.5 ± 1.1[a]	85.8 ± 9.6[a]	22.6 ± 0.5[a]	27.8 ± 4.1[a]	51.8 ± 0.5[a]	21.9 ± 0.4[a]	50.7 ± 1.3[a]	1.73 ± 0.02[a]	33.2 ± 0.9[a]

SG – starch granule; T_g – glass transition temperature; TS – tensile strength; EB – elongation at break. *E* – Young's modulus; ΔE^*_{ab} – colour difference estimative; *WI* – whiteness index; *YI* – yellowness index. Values in each column with different letters are significantly different ($p < 0.05$).
SOURCE: The author (2018).

The thickness of the *C. longa* starch film (sample d) was significantly different from sample b (control sample - corn starch film). The difference of 0.05 mm can be attributed to the size of the starch granule, as determined by the electron microscope in the form of the micrograph (Fig. 3 – samples a and c) presented in Table 3. According to Lim and Jane (LIM; JANE, 1992), starch film thickness is strongly correlated to starch granule size; the smaller the starch granules the thicker the film, as was found in the present study. Different thicknesses were found by the authors (BITENCOURT et al., 2014; MUSSO; SALGADO; MAURI, 2016), who developed gelatin-based films with *C. longa* extracts with thicknesses ranging from 0.05 to 0.08 mm. These differences were attributed to the different base materials used to obtain the films. The SEM micrographs in the present study show that the *C. longa* film (sample d) had a smooth surface compared to the control film made from corn starch (sample b), which presented a rougher surface, with waves affecting its thickness. The homogeneous matrix shown by sample d is a quality indicator of its integrity

(MALI et al., 2002) and better mechanical properties were anticipated, as presented (TS – tensile strength, Table 3) in the mechanical analysis.

FIGURE 3. SEM MICROGRAPHS OF: SAMPLE A – CORN STARCH (1.000X); SAMPLE B – CORN STARCH FILM (1.000X); SAMPLE C – NATIVE *CURCUMA LONGA* STARCH (4.500X); SAMPLE D – NATIVE *CURCUMA LONGA* STARCH FILM (1.000X).



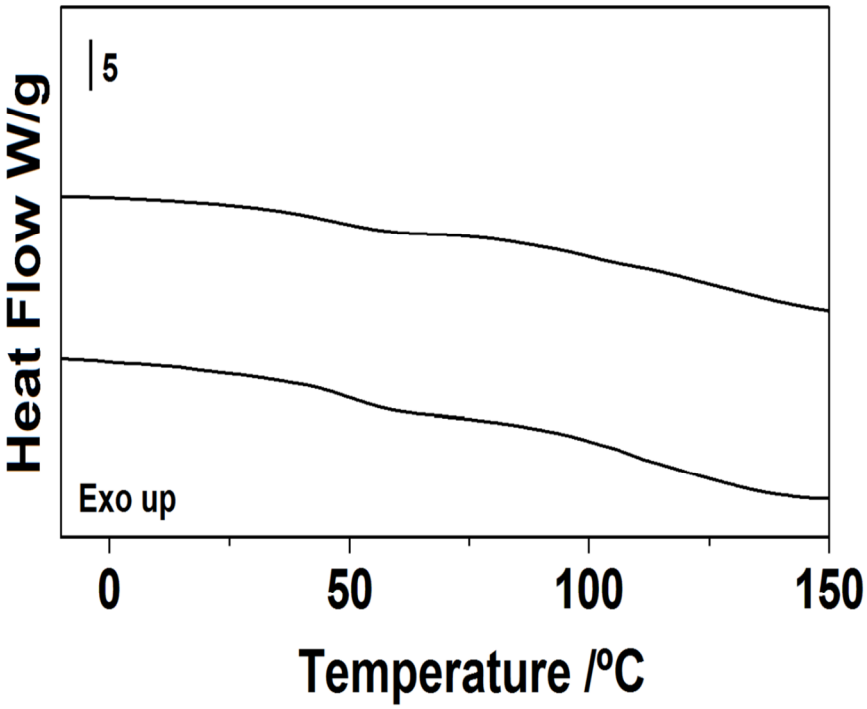
SOURCE: The author (2018).

3.5 Glass transition

An important parameter that can corroborate the performance of starch films (in addition to mechanical, storage and shelf life properties) is the glass transition temperature (T_g) (LI et al., 2015). The moment that the amorphous regions of a

polymer changes from the “glassy” to a “rubber” state (the softening point) is related to the T_g temperature. This moment, when coatings and films are more permeable to water vapour and oxygen, can cause the deterioration of diverse products. The glass transition temperatures of the control film (sample b – corn starch film) and the *C. longa* starch film (sample d) are shown in Table 3. The T_g temperature of sample d did not differ from the control sample. Both had temperatures (≅ 48 ºC) that were considered as efficient to provide stability during storage and transportation (ARVANITOYANNIS; BILIADERIS, 1998; JIMÉNEZ et al., 2012). In comparison, the *C. longa* starch film (sample d) showed a higher T_g temperature and was more stable than films developed from other starches (corn - 35.12 ºC; cassava - 42.42 ºC; and yam - 39.26 ºC) (MALI et al., 2006). These differences can be attributed to the starch source, growing conditions and method of analysis.

FIGURE 4. DSC CURVES OF: SAMPLE B – CORN STARCH FILM AND SAMPLE D – NATIVE *CURCUMA LONGA* STARCH FILM.



SOURCE: The author (2018).

3.6 Mechanical properties

The mechanical characteristics of the corn starch control film (sample b) and the *C. longa* starch film (sample d) are shown in Table 3. It is desirable for polymeric films such as starch films to demonstrate high elongation at break (%) values since

they are related to their flexibility and degradability (LI et al., 2015). Moreover, the EB is mainly affected by the tensile strength (TS). Films with increased TS show poor EB results (BITENCOURT et al., 2014). However, the *C. longa* starch film (sample d) showed a higher tensile strength (85.8 MPa) than the control sample (sample b), and no difference was obtained for the EB (22.0% sample b; 22.6% sample d) parameter. Hence, the *C. longa* starch film had better resistance to tensile strength and therefore good flexibility for handling. Young's modulus results were slightly different between the analyzed samples. Bitencourt et al. (BITENCOURT et al., 2014) obtained a maximum TS (MPa) of 35.1 and EB (%) of 36.5 for gelatin-based films with added curcuma ethanol extracts. Kalaycıoğlu et al. (KALAYCIOĞLU et al., 2017) produced chitosan films with incorporated turmeric extract (from commercial *C. longa* powder) that showed TS of 47.9 MPa and EB of 6.2 %. Comparing the current literature on different films, using *C. longa* starch to obtain bioactive films is more advantageous because it uses a single matrix, which can provide enhanced mechanical properties and antioxidant activity, as demonstrated in this study.

3.7 Colour analysis

Colour is a critical attribute for food products: it defines quality (freshness, maturity, and desirability) and acts as a barrier to the oxidation of lipids by light (BITENCOURT et al., 2014; HORNUNG et al., 2017). A CIE-lab scale was used to assess the degree of lightness (*L**), in which 0 represents black to 100, which represents whiteness; redness (*+a**) or greenness (*-a**); and yellowness (*+b**) or blueness (*-b**) of the film samples b and d, as shown in Table 3. As was visually perceived, and was also confirmed by the values obtained in the colour analysis, the *C. longa* starch film (sample d – Fig. 1) had a reddish yellow colour (*+a** - 21.9; *+b** - 50.7). This colouration can be attributed to the presence of the curcuminoids pigments of the *C. longa* rhizome, which were carried along during the starch extraction. The pigments persisted after the gelatinisation process to obtain the film, thereby corroborating with the report by Bitencourt et al. (BITENCOURT et al., 2014) that active films have natural pigments which can be expressed as coloured compounds.

4 Conclusions

The application of turmeric starch as a bioactive film provides a use for curcumin extraction residue (starch) as a reasonable alternative to synthetic plastics, which contribute to the accumulation of waste in the environment. Using HPLC-MS it was possible to identify and quantify four phenolic compounds from the *Curcuma longa L.* starch, which were also present in the film. It was verified that the starch and the film's total phenolic content (TPC) were significantly high when compared to the literature, and that they exhibited excellent antioxidant capacity, principally by donating or stabilising ions (DPPH and ABTS). The *C. longa* starch film showed a smooth and homogenous surface (observed in the SEM micrograph), a high glass transition temperature, strong resistance to tension and desirable extensibility, as well as a strong tendency to red and yellow colouration. Based on these findings, a single application of the starch from *Curcuma longa* L. resulted in a functional film that could provide efficient protection against microbial growth and oxidative deterioration in food products.

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FINAL CONSIDERATIONS

In this work the native and modified starch from three yam species (*Dioscorea sp.*; *Dioscorea piperifolia*; *Dioscorea trífida*) and from the turmeric rhizome (*Curcuma longa* L.), were characterized and applied as starch films and their properties were also investigated.

When comparing the *D. sp.* and *D. pipperifolia* native starches with potato, cassava and corn commercial starches it was found that they present higher resistance to thermal degradation and important paste properties correlated to their granules crystallinity, shape and size. In the natural modification developed to enhance the Dioscoreaceas (*Dioscorea sp.*; *Dioscorea piperifolia*) native starches including a different specie *Dioscorea trífida*, through the mixing properties it was obtained starches with improved technological features highlighting the high decrease of the enthalpy to form the starch gel and its tendency to retrogradation. These characteristics are required in a wide variety of industrial processes as desirable performance.

The native and the modified Dioscorea starches improved the properties of the biodegradable films developed showing high transparency and high solubility desirable for biodegradability and enhanced mechanical properties like high resistance to tensile strength. Thus, the yam starch mixtures formed strong and flexible films suitable for various industrial products through a simple green process.

Lastly, the native turmeric (*Curcuma longa* L.) starch investigated performed as a natural bioactive starch film presenting phenolic compounds and antioxidant activity. Furthermore, *C. longa* film exhibited a smooth structural surface and strong resistance to tensile force superior to the ones developed from the *Dioscorea* starches, as well as maintaining its elasticity. Therefore, a novel biodegradable starch film was obtained using the turmeric starch.

In conclusion, the studies presented in this thesis, advance the scientific knowledge on natural starches characterization and products derived from environmentally friendly processes.

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Author: Polyanna Silveira Hornung,Suelen Ávila,Marcelo Lazzarotto,Simone Rosa da Silveira Lazzarotto,Geisa Liandra de Andrade de Siqueira,Egon Schnitzler,Rosemary Hoffmann Ribani

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