WOLFAPPLE (*SOLANUM LYCOCARPUM* ST. HILL) STARCH: CHEMICAL AND PHYSICAL PROPERTIES
FRUTA-DO-LOBO (*SOLANUM LYCOCARPUM* ST. HILL): PROPRIEDADES QUÍMICAS E FÍSICAS DO AMIDO.

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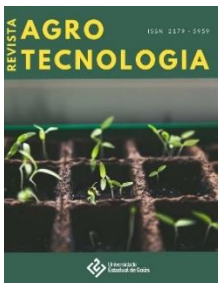

Abstract: In this study, starch from *S. lycocarpum* fruit (wolfapple) was extracted to determine physical and chemical properties and provide more information and further evidence on its characteristics. Starch yield was calculated. The chemical composition of the wolfapple starch was determined for ash, ether extract, fiber, protein and amylose content. The shape and size of the granules were assessed, as well as rapid visco analysis (RVA); differential scanning calorimetry (DSC); and crystallographic analysis. The yield of the wolfapple starch was 27.9% and the chemical composition was characterized by the presence of 0.19% ± 0.01 ash, 0.07% ± 0.00 ether extract, 0.32 % ± 0.01% proteins, 0.03% ± 0.01 fibers, and 29.16% ± 0.09 amylose. Starch image analysis presented conical shaped granules with average diameters and respective standard deviations of 16.59 ± 3.68 and 16.52 ± 3.36 μm, for the largest and smallest diameter, respectively. Paste viscosity, calorimetric, and crystallographic analyses showed that the starch from wolfapple has desirable characteristics such as stability at high temperature, making it a promising material in various food and industrial sectors.

KEYWORDS: Amylose, gelatinization, starch granule.

Resumo: O objetivo deste trabalho foi extrair e determinar as propriedades físicas e químicas do amido da fruta de *S. lycocarpum* (fruta-do-lobo) visando obter e disponibilizar mais informações sobre sua caracterização. Para isto, realizou-se extração do amido e o cálculo do seu rendimento. Determinou-se também sua composição química (cinzas, extrato etéreo, fibras, proteínas e teor de amilose); forma e tamanho dos grânulos; análise de viscosidade (RVA); análise calorimétrica (DSC) e análise cristalográfica. A extração do amido da fruta de *S. lycocarpum* teve um rendimento de 27,9% e este se caracterizou por apresentar 0,19% ±0,01 de cinzas, 0,07% ± 0,00 de extrato etéreo, 0,32% ± 0,01de proteínas, 0,03% ± 0,01 de fibras e 29,16% ± 0,09 de amilose. A análise de imagem do amido mostrou grânulos com formato cônico e diâmetros médios e respectivos desvios padrão de 16,59±3,68 e 16,52±3,36 μm, para diâmetro maior e menor, respectivamente. As análises da viscosidade de pasta; calorimétrica e cristalográfica revelou que o amido da fruta de *S. lycocarpum* mostrou perfil com características desejáveis, como boa estabilidade a alta temperatura, o que o elege como um bom ingrediente a ser usado em diversos setores alimentícios e industriais.

PALAVRAS-CHAVE: Amilose, gelatinização, grânulos de amido.

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INTRODUCTION

Starch is a plant-produced polymer stored in small and semicrystalline granules composed by two main components: amylose and amylopectin. Amylose predominantly consists of linear chains, typically consisting of α (1-4) D-glucose units, while amylopectin has large and branched chains formed by α (1-4) D-glucose units in the rectilinear portions and α bonds (1-6) in the branches (BENINCA et al., 2013). It is the main source of reserve carbohydrate in plants and an important source of energy for many organisms, providing between 70 to 80% of the calories consumed by humans (SPIER, 2010). Starch can be extracted from cereal grains, vegetables and tubers, and has a variety of food and non-food applications (SCHIRMER et al., 2013; FERNANDES et al., 2019).

The starch industry extracts starch from various sources and processes it into a wide variety of products, such as native starches, glucose, maltose and fructose syrups, dextrose and maltodextrins (CEREDA, 2002). Figure 1 shows some applications of starch, both in natural and modified forms.

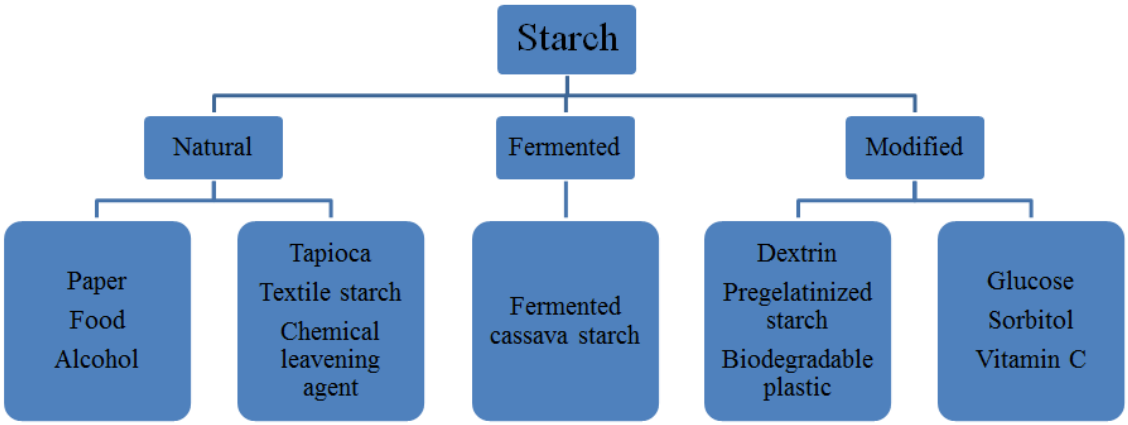

Figure 1. Main applications of starch in Brazil.
Source: Adapted from Beninca et al, 2013.

Among other usages in the food industries, starch can facilitate food processing and is used as thickener in soups, broths, and meat sauces. It provides suspended solids and texture, gelling agents in cured processed meat, stabilizers in salad dressings, and increase the shelf life of products (CEREDA, 2002). Starch is also used due to its viscosity, gelling power, adhesion, tendency to retrograde, among other properties affected by the amylose/amylopectin ratio as well as the shape and size of granules (CIACCO; CRUZ, 1987).

In the textile industry, starch is used for gumming threads to facilitate weaving

and in papermaking to produce papers of different strengths and high print quality (BENINCA, et al., 2013). In glue and adhesive manufacturing, in general, it is used for providing more viscous products, for its easy preparation and for combining with various resins and synthetic emulsions (CIACCO; CRUZ, 1987). It can be used in the composition of biodegradable packaging (FERNANDES et al., 2019). In culture media, starch can be useful as an inductor of the production of enzymes and microbial biomass. In addition, it can be hydrolyzed and the sugar produced from hydrolysis can be fermented to produce ethanol used for food, beverage, pharmacological and laboratory purposes (AJIBOLA et al., 2012). The glucose obtained from starch is also widely used in the manufacture of organic acids such as citric acid, glutamic acid, lactic acid, and amino acids such as lysine, or commercialized as crystalline glucose. Therefore, it is very important to have different starch sources available for this very wide market (AQUINO et al., 2015).

The most prominent starchy species in the world market are corn, potato, and cassava. However, recent studies have searched for new sources of starch with potential to be used in various sectors (DHANAPAL et al., 2012; KASIRAJAN; NGOUAJIO, 2012). Industrial applications consider availability and physicochemical characteristics of the starch, which may vary according to its origin. Thus, every natural or native starch is considered unique (MATSUGUMA et al., 2009).

The *Cerrado* region in Brazil has a variety of plants with very specific characteristics due to their adaptations to the climate and soil. These plants are still little explored and could be used as a source of starch. *Solanum lycocarpum* St. Hill , commonly known as wolfapple, belongs to the family Solanaceae, the most common genus found in the Brazilian *Cerrado* (Figure 2). It can produce from 40 to 100 fruits, of masses varying between 400 to 900g, with good potential for starch extraction (CAMPOS, 1994; FERNANDES et al.; 2019).

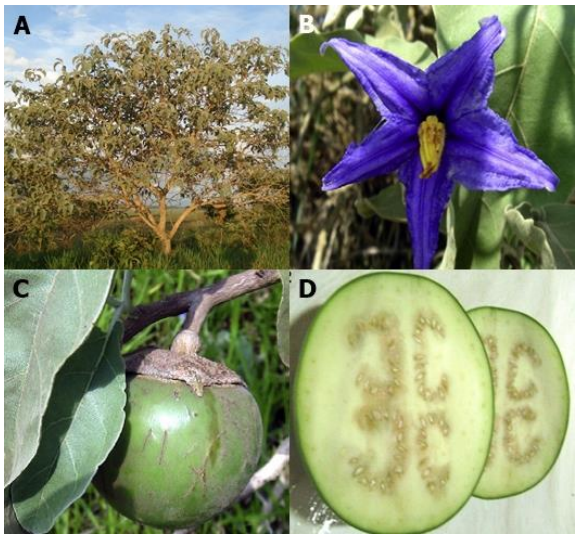

Figure 2. *S. lycocarpum* a) plant; b) flower; c) and d) fruit cross section.
Source: Adapted from Campos (1994).

In the present study, starch from *S. lycocarpum* fruit (wolfapple) was extracted to determine physical and chemical properties and provide more information and further evidence on its characteristics.

MATERIAL AND METHODS

S. lycocarpum fruits were collected in the rural area of the municipality of Anápolis-GO. Pulp samples were collected in paper bags and stored in a refrigerator for starch extraction.

To obtain the starch, the pulp of the peeled fruits was cut into small pieces with a stainless steel knife and stored in plastic buckets containing a 5 $g.L^{-1}$ sodium metabisulphite solution to prevent browning.

The pulp was milled in a "CROTON" MA580 knife mill (MARCONI, Piracicaba, Brazil) and sieved to 10 mesh (1700 µm). The pulp was again sieved (150 to 400 mesh), and repeatedly washed under running water for starch separation and decantation in plastic containers. Following decantation, the material was washed with absolute alcohol to remove resins and vacuum filtered. After purification, the starch was vacuum dried at 40°C to constant weight. The dehydrated starch was kept in a desiccator containing silica gel for 12h and reduced to powder using mortar and pestle. Finally, the material was sieved to 48 mesh to obtain the dry sample for analysis.

The yield was calculated as the ratio between the weight of the peeled pulp and the starch produced. The material was characterized considering ash, fiber, ether extract, and protein content (AOAC, 2000).

Amylose content was determined according to the methodology proposed by Williams et al. (1970).

Image analysis was used to evaluate the shape and size of the isolated starch granules. The samples were collected with platinum wire on a glass slide, mixed with a drop of distilled water, and covered with a coverslip. The slides were examined with a LEICA DME optical microscope (Wetzlar, Germany), and the selected images were analyzed with the software LAS EZ.

The methodology described by Ascheri et al. (2006) was used for viscosity analysis, using the Rapid Visco Analyzer (RVA). Starch suspensions (2.5 g in 25 mL of water) were corrected to 14% moisture and assessed using the following time/temperature regime: one minute at 50ºC , heating from 50 to 95°C at a rate of 6ºC.min^{-1}, holding at 95°C for 5 minutes and cooling from 95 to 50°C at a rate of 6ºC.min^{-1}. Viscosity was expressed as cP. Based on the graph obtained, the following parameters were evaluated: pasting temperature, maximum viscosity (peak), viscosity decrease (difference between maximum viscosity and viscosity at 95°C for 5 min), final viscosity, and retrogradation tendency (difference between final viscosity and viscosity at 95°C for 5 min).

Calorimetric analysis was performed on a DSC Q200 differential scanning calorimeter (TA Instruments, New Castle, USA), using indium calibration. To determine the gelatinization temperature, approximately 5.0 mg of the sample (at known moisture) was placed into an airtight aluminum container. The scanning profile used was balanced at 5ºC, heating at 110 ºC, heating rate of 10ºC.min^{-1}, and flow of 50 mL nitrogen per minute. Gelatinization enthalpy was determined using the software Universal Analysis version 4.3A.

Crystallographic analysis was carried out with the samples fixed in an aluminum support and assessed at room temperature, using a Rigaku X-ray diffractometer (model RU 200 R, Osaka, Japan), operating with a monochromatic filter, copper Ka radiation, 0.8 kW power, 50 mA current, 40 kV voltage and wavelength of 1.54 A. The analyses were performed between $2\theta = 5^{o}$ and $2\theta = 40^{o}$, with scanning speed of $2^{o}(2\theta).min^{-1}$, and intensity expressed as peak count per second.

RESULTS AND DISCUSSIONS

Yield of starch extracted from *S. lycocarpum* was 27.9% on a dry matter basis. Although this value is lower than that found by Di-Medeiros et al. (2014) for the same botanical source (51%), still this yield is considered good when compared with

other starch sources such as the commercial starch from cassava roots, which can range between 21-33% (dry basis) (CEREDA, 2002). According to Lima et al. (2012), easiness to extract starch depends on granule size and other factors. *S. lycocarpum* fruit starch has large granules that facilitate decantation and extraction efficiency.

Difference between the yields reported are due the methodologies used in the extraction processes.

The results of centesimal composition (Table 1) were within the required by the Brazilian legislation for starches extracted from natural sources. They indicate a high purity level of the *S. lycocarpum* fruit starch, with low contents of ash, ether extract, fiber, and protein.

Table 1 indicates that the starch from *S. lycocarpum* fruit has 29.16% of amylose in its content. This value is considered high and suggests it can be used in various industrial fields, like production of films, biomembranes of low water permeability, food additives, or pharmaceutical coating materials (Di-Medeiros et al, 2014).

Table 1. Chemical composition of *S. lycocarpum* fruit starch.

Constituent	Mean ± Standard Deviation (% dry matter basis)
Ash	0.19 ± 0.01
Ether Extrato	0.07 ± 0.00
Protein	0.32 ± 0.01
Fiber	0.03 ± 0.01
Amylose	29.16 ± 0.09

Each value represents the mean and standard deviation of three repetitions.

The image analysis (Figure 3) showed conical shaped granules, mean diameters and standard deviations of 16.59 ± 3.68 and 16.52 ± 3.36 µm, for larger and smaller diameters, respectively.

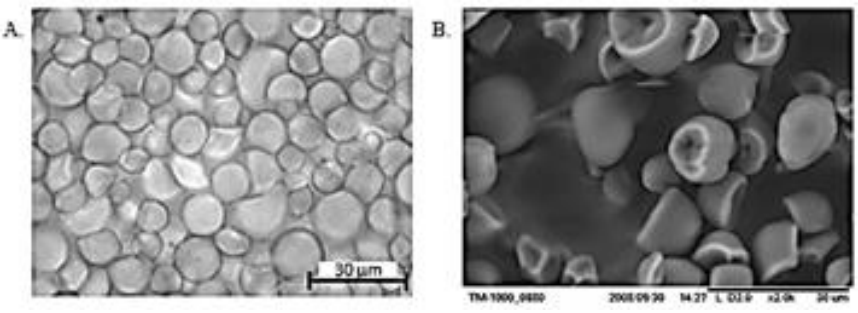

Figure 3. (A) Optical photomicrograph (x100) and (B) Scanning electron microscope (SEM) (x2000) granule diameters of *S. lycocarpum* fruit starch .

Eliasson (1996) classified starch granules of different botanical sources as small granules for sizes ranging from 1 to 10 µm and large granules for sizes ranging

from 10 to 35 µm. Di-medeiros et al. (2014), studying the rheological and biochemical properties of *S. lycocarpum* starch also found conical shaped granules, but with average sizes from 10 to 14.4 µm. This variation can occur because the granule size and amylose content of starches of the same species may vary due to the environmental conditions of plant growth (CIACCO; CRUZ, 1987).

Eliasson (1996) discussed that the size of granules and their distribution are among the factors that influence the functional properties of starches. In addition, it is an important parameter to define the steps in the extraction process of this polysaccharide. The extraction process with large granules, for example, is more efficient due to the easiness of decantation and extraction.

The viscosity profile of the starch paste from *S. lycocarpum* is shown in Figure 4. Paste temperature and the viscosity peaked at 69.5°C and 4,461 cP, at the time of 2.6 min and 5.7 min, respectively. Viscosity was stable at hot temperatures with a slight decrease after reaching the maximum viscosity peak.

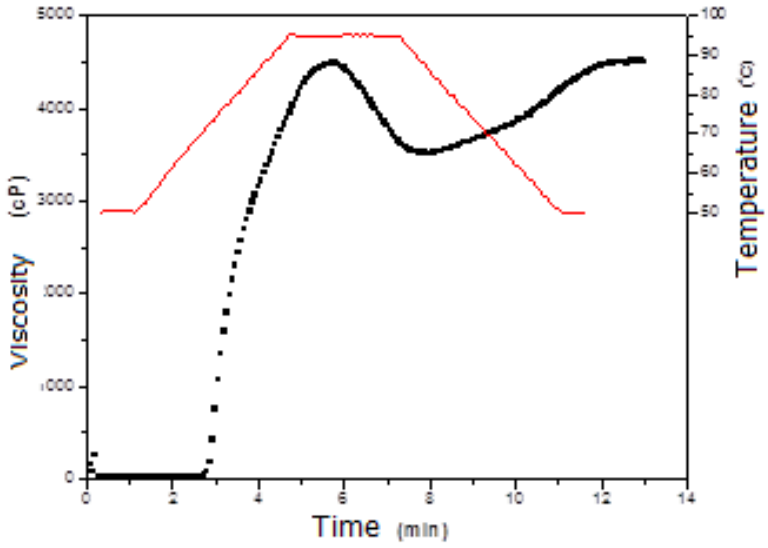

Figure 4. Viscosity profile of the starch paste from *S. lycocarpum* fruit.

The viscosity peak of *S. lycocarpum* starch showed a round shape, indicating heterogeneous starch granules. After viscosity rupture and subsequent cooling, there was a retrogradation tendency of 923.5 cP, reaching final viscosity of 4438 cP.

Retrogradation or recrystallization is a process that occurs after solubilization and during gelatinization. The amylose chains aggregate faster than the amylopectin and

form crystalline double helices stabilized by hydrogen bonds. Upon cooling and/or staling processes, these helices form highly stabilized three-dimensional crystalline structures (ELIASSON, 1996).

The viscosity profile in Figure 4 shows that the starch from *S. lycocarpum* fruit has desirable features for the production of biodegradable films: good stability at high temperature and mechanical stability (FERNANDES et al., 2019). In addition, it is also a good material for the processed food industry, as well as in the adhesive industry (CEREDA 2002; DI-MEDEIROS et al. 2014).

Considering the calorimetric analysis of starches, Di-Medeiros et al. (2014) stated that this property is very important to determine the functionality and utilization of starch in the industry. The gelatinization properties of the starch from *S. lycocarpum* and other botanical sources are presented in Table 2.

Table 2. Starch gelatinization properties of wolfapple (*S. lycocarpum*) and other botanical sources.

	Gelatinization			
	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (J.g^{-1})
Wolfapple (*S. lycocarpum*)	63.68± 0.27	67.20 ± 0.06	77.81 ± 0.28	13.08 ± 0.72
Cassava (*Manihot esculenta*)*	61.15 ± 0.18	67.73 ± 0.35	73.95 ± 0.17	13.73 ± 1.33
Edible Canas (*Canna edulis*)*	65.44 ± 0.05	70.08 ± 0.01	74.84 ± 0.09	14.24 ± 0.72
Turmeric (*Curcuma longa*)*	78.78 ± 0.18	82.68 ± 0.25	89.04 ± 0.55	13.73 ± 0.24
Ginger (*Zingiber officinale*)*	81.77 ± 0.28	87.41 ± 0.35	93.28 ± 0.56	20.23 ± 1.30

* Source: Peroni (2003), T_0 = initial temperature, T_p = peak temperature, T_c = conclusion temperature and ΔH = enthalpy variation.

Comparison of the initial gelatinization temperatures (T_0) obtained from DSC with the paste temperatures of RVA (Figure 4) shows that the initial gelatinization temperature of the *S. lycocarpum* starch is lower than that of the paste temperature (69.5°C). The same result was found by Peroni (2003) for cassava (*Manihot esculenta*), Edible Cana (*Canna edulis*), turmeric (*Curcuma longa*), and ginger (*Zingiber officinale*) starches, which present higher values than those obtained by DSC. According to Cereda (2002), the paste temperature obtained by RVA was higher

because it is sensitive to the first increases in the starch paste viscosity, which is different from the initial gelatinization temperature detected when the first granules begin to disrupt. DSC values are more accurate, while RVA values can show temperature ranges.

Yoo and Jane (2002) showed that a single endotherm found for starches means that amylose is not complexed with lipids, since, if present, they could fuse and produce an endotherm within the temperature range of 91-100 °C. This is in agreement with the findings of this study showing low fat content (0.07%) in *S. lycocarpum* starch (Table 1). The value of this endotherm was 13.08 $J.g^{-1}$ (Figure 5).

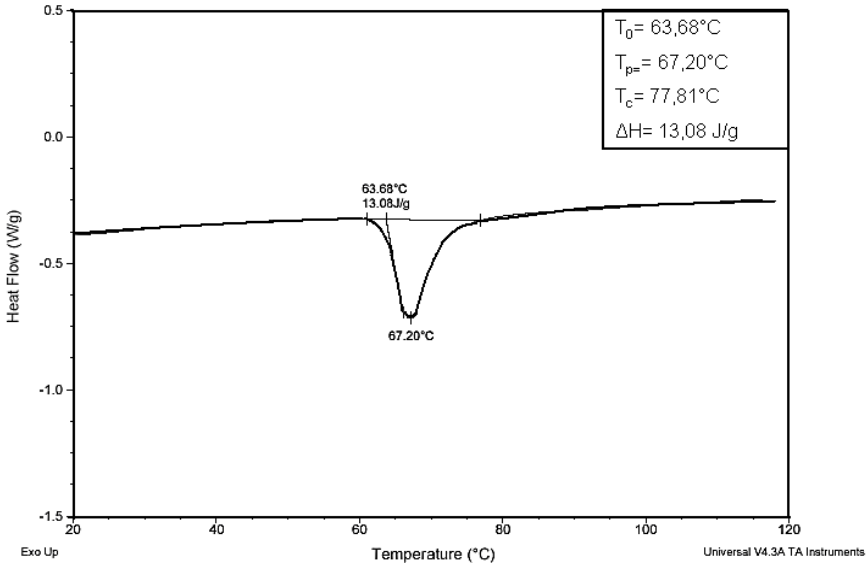

Figure 5. DSC thermogram for *S. lycocarpum* starch.

The peak in the DSC curve pointing down in Figure 5 is a representation of the endothermic gelatinization reaction that involves fusion of starch granules. According Cereda (2002), T_0, Tp, and Tc are influenced by the molecular structure of the crystalline region, which corresponds to the distribution of short chains of amylopectin.

ΔH gel may be correlated with both amylopectin crystallinity and the strength with which the double helices formed by their chains are associated with the starch granule. Thus, the higher the ΔH gel, the greater the force required to break the granule structure, resulting in gelatinization. In thie study, *S. Lycocarpum* starch required 13.08$J.g^{-1}$, which is

considered high and indicates a strong association of the amylopectin.

A good gelatinization temperature range (R = (Tc - To)) was found for starch of *S. lycocarpum* fruit, suggesting the presence of crystals with different stability within the crystalline zone, which are broken at different temperatures (Cereda, 2002).

Abraharm and Simi (2008) discussed that starch gel gelatinization is a response to the nature of the granules, regarding their amylose and amylopectin contents, the spatial arrangement of both polysaccharides in the internal structure of the granule, and the degree of compaction. The starch gelatinization properties of *S. lycocarpum* confirm its potential in the production of biodegradable films, having potential for coating of agricultural seeds that require rapid germination (FERNANDES et al., 2019).

Cereda (2002) and Ciacco and Cruz (1987) found that crystallinity patterns are defined based on the interplanar spaces (d) and the relative intensity of the X-ray diffraction lines. The starch from *S. lycocarpum* produced six main peaks around the diffraction angles 4.2°, 6.5°, 14.8°, 17.2°, 21.5° and 23.8° (Figure 6). These peaks are typical of granules with type B structure, presenting few branched chains and numerous long chains.

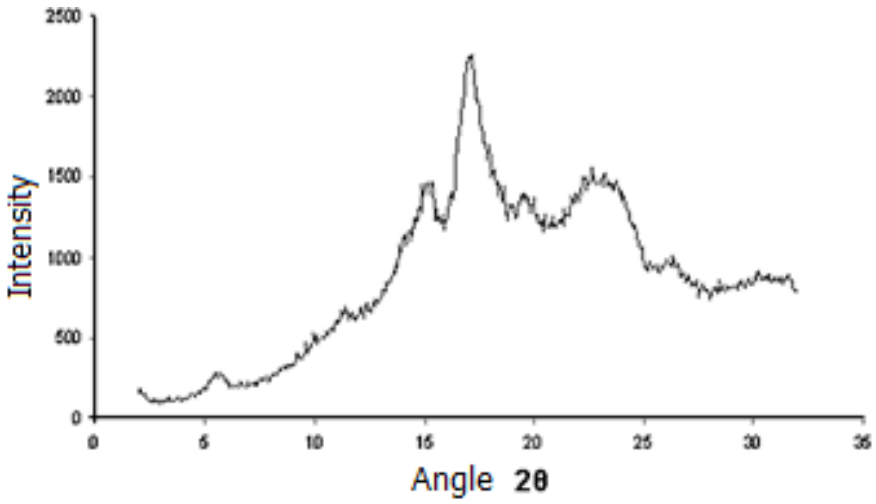

Figure 6. X-ray diffractogram of *S. lycocarpum* starch.

Table 3 shows the results for the angle 2θ, the interplanar space, and intensity of *S. lycocarpum* and *Arracacia xanthorrhiza* starches.

Table 3. Main x-ray diffraction intensity peaks of starch from *S. lycocarpum* and *A. Xanthorrhiza*.

Solanum lycocarpum			*Arracacia Xanthorrhiza**		
2θ	Intensity	d**	2θ	Intensity	d**
6.55	192	13.48	5.55	146	15.90
14.8	1272	5.98	14.9	266	5.94
17.15	2253	5.16	17.02	635	5.20
19.1	1239	4.64	19.27	240	4.60
21.45	1219	4.14	22.03	331	4.03
23.75	1479	3.74	24.0	256	3.70

* Source: VIEIRA (2004)
** Calculated with Bragg's law.

In the characterization by x-ray diffraction of *Arracacia xanthorrhiza* starch, Vieira (2004) found similar values for the six main peaks around the diffraction angles, showing that the starch is also type B, but the intensities found for the respective peaks were much lower.

Type B crystal starch has an open and highly hydrated structure with double helices arranged in a hexagonal fashion (CEREDA, 2002). It is found in the starch from tubers, which are varieties with high amylose content and retrograded starches. This is in agreement with the high amylose content found for *S. lycocarpum* (Table 1) in the present study. Lajolo and Menezes (2006) also agree that because they are rich in amylose, these types of starch present similar shapes and sizes and are resistant to both enzymatic and acid hydrolysis, which makes them comparable to dietary fibers. This characteristic increases the potential use of this raw material in the food industry. However, further and more detailed research is needed to evaluate the presence of substances unsuitable for food consumption (LAJOLO; MENEZES, 2006).

CONCLUSIONS

S. lycocarpum fruits have potential as a raw material for starch extraction as they produce yields comparable to that of raw materials from commercial starch sources.

Chemical composition, paste viscosity, calorimetric and crystallographic analyses showed that starch from *S. lycocarpum* has desirable characteristics: good stability at high temperature and mechanical stability, which makes it a promising material for several food and industrial applications.

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