Calcium oxalate content from two Amazonian amilaceous roots and the functional properties of their isolated starches

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Abstract
The calcium oxalate content from two Amazonian amilaceous roots, Xanthosoma sagittifolium and Pachyrhizus tuberosus, before and after cooking were studied in the present work. Calcium oxalate raphides were observed by light microscopy in longitudinal sections from fresh tubers. The functional properties and physicochemical characteristics of isolated starches from both species were also investigated. Starches granules were observed with scanning electronic microscopy and presented a typical oval-circular shape, smooth surfaces and size ranging from 3.6 μm to 14 μm. Lower gel clarity, slightly high water-binding capacity, A-type starch structure and low values of crystallinity degree were determined in the isolated starches. High intensity bands related to vibrations of glucosidic bonds of polysaccharides were observed by infrared spectroscopy in both starches. Thermal degradation behavior data of the isolated starches revealed high initial decomposition temperatures. Thus, the studied cooked tubers presented low levels of calcium oxalates and are safe to be consumed as a boiled vegetable. Their isolated starches possess good functional characteristics to be applied as an ingredient to products in food industry.

Keywords: Xanthosoma sagittifoilum; Pachyrhizus tuberosus; edible tubers; starch; calcium oxalate.

Practical Application: Application as a cassava starch substitute and development of vegan food products.

1 Introduction
Cocoyam, taro, aerial yams and other tuberous roots are nutritive and relevant crops to several tropical countries in South America, Africa, Asia and Oceania (Londoño-Restrepo et al., 2018; Otegbayo et al., 2014). Tubers are subsistence foods and rich sources of energy to human diet, especially for those who have poor access to other foods resources. These amilaceous food are vital crops to indigenous communities and are related to their culture and traditions (Boakye et al., 2018). Tuberous roots can be consumed as cooked vegetables, as a flour or as an isolated starch (Uchechukwu-Agua et al., 2015). Starches are a diverse and useful ingredient for industrial food applications, such as thickeners, fillers, gelling and water holding agents (Odeku, 2013; Zhu, 2016). Researches about starch sources from untraditional tuberous roots has raised due to their functional properties and to the intense demand of carbohydrates in food and pharmaceutical industries (Londoño-Restrepo et al., 2014; Odeku, 2013). Starch properties, such as, shape and granules size, as well as its crystallinity pattern, water binding capacity and thomodegradation behavior, are important factors to be considered when applied into products (Jayakody et al., 2007).

Tayobe or Cocoyam belongs to the Araceae family, which the Xanthosoma is a significant genus used as food, even though commercially underexplored (Falade & Dkafor, 2013). This genus comprises more than 50 species, with special emphasis to X. sagittifolium (L.) Schott, which is one of the most economically important (Boakye et al., 2018). The nutritional profile of this tuber is related to carbohydrates (33-36 g/100g), proteins (3.9-5.5 g/100g) and fibers (1.1-1.7 g/100g) (Sefa-Dedeh & Agyir-Sackey, 2004). However, due to oxalates content in all parts of X. sagittifolium, which reflects in acridity taste and irritative reactions, their fresh consumption is limited (Boakye et al., 2018; Sefa-Dedeh & Agyir-Sackey, 2004). Their tubers are consumed as a boiled vegetable, as a flour, and as starch (Graf et al., 2018). Starches from X. sagittifolium presented a Lego-like shape with diameters ranging from 8 to 35 μm, an A-type diffraction pattern and a crystallinity of 45% (Graf et al., 2018; Zhu, 2016).

Another edible tuberous root native from South and Central America is the genus Pachyrhizus, commonly named as yam bean or jacatupé (Forsyth et al., 2002; Ramos-de-la-Peña et al., 2013). This genus belongs to the botanical family of Fabaceae (Díaz et al., 2016). The major nutrients present in the tuber of Pachyrhizus ssp. are carbohydrates (47.8-65.0%) and proteins (7.4-11.5%) as reported by some authors (Ascheri et al., 2014; Díaz et al., 2016; Ramos-de-la-Peña et al., 2013). High carbohydrates and fibers, as well as vitamins and minerals contributed to classify this tuber as a health energy food (Ramos-de-la-Peña et al., 2013). A high starch content, approximately 83% in dry matter, plus a significant production yield of 5.0 kg/m² (Ramos-de-la-Peña et al., 2013) contributes to starch agroindustry, which may turn P. tuberosus an attractive resource for the sector. Pachyrhizus ssp starch revealed a circular and polygonal microstructural shape of 10-25 μm, and low pasting temperature of 56 °C (Ramos-de-la-Peña et al., 2013).
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2 Materials and methods

2.1 Materials

Fresh roots from *Xanthosoma sagittifolium* (XA) and *Pachyrhizus tuberosus* (PT) were collected in the farm from the Federal University of Amazonas. The roots were cleaned and washed with distilled water to remove physical dirt. The washed roots were stored under refrigeration temperature until use. All reagents used were analytical grade.

2.2 Starch extraction

The starches from *Xanthosoma sagittifolium* and *Pachyrhizus tuberosus*, named as XAS and PTS, respectively, were extracted using methodology adapted from (Ascheri et al., 2014). Successive washes followed by decantation and filtration with a polyester mesh (40 cm x 40 cm) at room temperature were realized until the samples were visually appearing a starch. Subsequently, it was air dried (Solab, model SL-100, SP, Brazil) at 50 °C for 12h and passed through a 48 Mesh sieve to homogenize the samples.

2.3 Proximate composition analysis of raw material

The proximate composition of XA and PT samples was determined following the methods of Association of Official Analytical Chemists (2006). Moisture (method 934.06), crude protein (method 990.03), crude fiber (Method 978.10), total fat (method 983.23) and ash contents (method 942.05). The carbohydrate content was estimated by difference. Analysis were conducted in triplicate.

2.4 Microstructure and morphology observations

In order to investigate the presence of calcium oxalate raphides in XA and PT rhizomes an optical microscope Primo Star (Carl Zeiss, Jena, Germany) was used. Cross and longitudinal sections in the direction of the roots were made freehand with stainless steel blades. The anatomical investigation was performed with XA and PT fresh rhizomes. Thin sections were gently placed on glass slides with a drop of distilled water and glass covered for observation. The external morphology, shape and distribution of XAS and PTS granules were observed by scanning electron microscopy (SEM). XAS and PTS powder were sprinkled onto double-sided adhesive tape, attached to circular specimen stubs, coated with silver under 6 x 10^1 mbar ultrapure argon vacuum from Baltec (SCD 005 Sputter Coater, Balzers, Liechtenstein) for 150s, examined at 5 kV and photographed (TM3030 Plus, Hitachi, Tokyo, Japan).

2.5 Thermal processing and drying conditions

The tuber samples were peeled off and manual sliced into approximately 2 cm x 2 cm thickness using a stainless-steel knife. The XA and PT samples were separated in two groups, uncooked and cooked. The samples were cooked in boiling water for 40 min (time previously tested as sufficient to obtain soft roots for consumption). Uncooked and cooked slices samples were air-dried at 105 °C until constant weight. Dried samples were separated, milled in domestic blender and passed through a 0.5 mm sieve. The milled samples were stored in air-tight containers prior to the determination of their anti-nutrient compositions.

2.6 Anti-nutrients contents before and after thermal processing

Anti-nutrients were determined for the uncooked and cooked samples, with methodology from Kumar et al. (2017) and expressed as calcium oxalate content. The calcium oxalate content was investigated by titration a previously acidified aliquot of the samples with 3 N H₂SO₄ against 0.1 N KMnO₄ solutions under 90 °C, until a pink color persisted. The procedure was repeated in triplicate.

2.7 Functional properties of the starches

Water-binding capacity, pH and gel clarity

WBC of XAS and PTS was gravimetrically measured according to methodology from Jiang et al. (2012). The WBC was calculated with the following Equation 1:

\[
WBC = \left( \frac{W_f - W_0}{W_0} \right) \times 100
\]

where \(W_0\) is the initial weight of the dried starch samples, and \(W_f\) is the weight of swollen starch samples. The pH of 1% starch/distilled water suspensions was measured using a digital pH meter. Gel clarity was expressed as light transmittance percentage (T%) and measured in triplicate at 650 nm with a Cary 100 spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA) according to Craig et al. (1989).

Crystallinity degree

The crystallinity degree (CrD) of XAS and PTS was analyzed with an X-Ray diffractometer (Ultima IV, Rigaku Corporation, Tokyo, Japan) in the angular region 2° to 50° (2θ) at 0.05° per second (2θ) in continuous scanning mode with CuKα radiation generated at 40 kV and 20 mA. CrD of samples was calculated as described by Bendaoud et al. (2017).

Fourier transform infrared spectroscopy

Infrared spectroscopy (FTIR) was used to evaluate the molecular structure of XAS and PTS. A Frontier FTIR/FIR spectrophotometer (model 98737, Perkin Elmer Spectrum, CT, USA) equipped with a Universal Attenuated Total Reflectance (ATR) cell device was used and the analysis were carried out from 4000-400 cm⁻¹ wavenumbers. All spectra were registered with a 4 cm⁻¹ resolution in transmission mode by averaging 60 scans and samples were analyzed as potassium bromide (KBr) disks.
**Thermal degradation**

Thermogravimetric (TG) and differential thermal analyses (DTA) were used to investigate the thermal degradation behavior of the XAS and PTS. The analyses were carried out under nitrogen atmosphere on TGA Q-500 equipment (TA Instruments, New Castle, USA). The samples (10 mg) were heated from 25 °C to 700 °C at a 10 °C/min.

**2.8 Statistical analysis**

Experimental data was submitted to variance analysis (ANOVA) and significant differences between means were established at p ≤ 0.05. Tukey's test was applied.

**3 Results and discussion**

**3.1 Proximate composition analysis of raw material**

Information about the nutritional composition of unusual tuberous roots, such as XA and PT, is important because of the common use of this food for the local communities in South America. The results of proximate composition from XA and PT are listed in Table 1. In general, XA and PT presented high moisture content, 77 and 89 g/100g of fresh sample, respectively. A similar value, 77 g/100g, of moisture were found in sweet potato (Uchechukwu-Agua et al., 2015). The major nutrient found in XA and PT was carbohydrates. Those were lower than the values (18-37 g/100g) determined for red-flesh X. sagittifolium (Sefa-Dedeh & Agyir-Sackey, 2004). The chemical composition of the studied tubers showed low contents of fats, crude fibers and ashes. The crude protein composition was significantly higher in XA than PT. The crude protein in PT was inferior to values (4.3-7.4%) quantified in other works with different phenotypes of P. tuberosus (Ascheri et al., 2014). The flour from X. robustum presented 6.8% of crude protein (Londoño-Restrepo et al., 2014). The major variety on the nutritional composition of these tubers are related to the differences between species, phenotypes and environmental growth conditions.

**3.2 Microstructure and morphology observations**

The light microscopic observations were used to visualize the presence of calcium oxalate raphides in sections of XA and PT fresh rhizomes. It was observed in XA and PT fresh rhizome sections, the presence of raphides aggregates with spherical shape, which is related to calcium oxalate as shown in Figure 1. The shape (needles, spherical aggregates, crystal sand, styloid, and prisms) or tissue locations of calcium oxalate crystals are related to growth conditions of each species (Ilarslan et al., 1997).

The morphology and distribution of XAS and PTS starch granules were observed by scanning electronic microscopy and are shown in Figure 2. The starch granules presented ovoid and circular shapes. The starch granules of both species showed dimensions varying from 3.6 μm to 14 μm and their surfaces are smooth. It was noted that the starch granules formed a Lego-like macro particles (Londoño-Restrepo et al., 2018), which exhibit irregular and dense shapes. Similar shapes and sizes were observed in Dioscorea opposita tuber starch (Chen et al., 2017a). The starch form X. robustum presented a circular shape and size between 8 and 35 μm (Londoño-Restrepo et al., 2014).

**3.3 Anti-nutrients contents before and after thermal processing**

The calcium oxalate content as an anti-nutrient in XA and PT were determined before and after thermal treatment. The results are listed in Table 2. The cooking effect promoted a relevant reduction of more than 85% in oxalate content for both tubers (p < 0.05). The uncooked XA and PT presented high content of calcium oxalate, when compared to the values determined for the cooked tubers. The cooking effect in the reduction of calcium oxalate content is in accordance to the records in the literature for boiled vegetables (Akpan & Umoh, 2004; Savage et al., 2000). Studies with two species of X. sagittifolium reported an oxalate content in fresh samples ranging from 254 to 460 µg/100g and a significant reduction

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**Table 1. Proximate composition of two Amazonian tubers.**

| Composition (g/100g)* | Xanthosoma sagittifolium | Pachyrhizus tuberosus |
|-----------------------|--------------------------|-----------------------|
| Moisture              | 77.1 ± 2.82<sup>a</sup>  | 89.0 ± 3.7<sup>b</sup> |
| Ashes                 | 1.1 ± 0.09<sup>a</sup>   | 0.6 ± 0.43<sup>a</sup> |
| Crude proteins        | 4.4 ± 0.42<sup>a</sup>   | 1.1 ± 0.23<sup>b</sup> |
| Total fats            | 0.2 ± 0.06<sup>a</sup>   | 0.1 ± 0.03<sup>a</sup> |
| Crude fibers          | 0.7 ± 0.07<sup>a</sup>   | 1.20 ± 0.08<sup>b</sup> |
| Carbohydrates         | 16.6 ± 3.48<sup>a</sup>  | 8.03 ± 4.30<sup>a</sup> |

Data presented as mean ± standard deviation (n = 3); *Dry basis (except moisture content); Lowercase letters <sup>a,b</sup> overwritten in the same line shows significant difference (p < 0.05) by Tukey's test.

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Figure 1. Calcium oxalate raphides in (a) Xanthosoma sagittifolium and (b) Pachyrhizus tuberosus fresh sections roots observed within a 40x magnification.
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3.4 Functional properties of the starches

Oxalate content reduction of 50% in this substance by drying it in double roller drum dryer (Sefa-Dedeh & Agyir-Sackey, 2004). The reduction of more than 70% of oxalate content in flours prepared with pre-cooked slices from Colocasia esculenta in lemon solution was also reported by Kumar et al. (2017). For these authors, the oxalates are related to acridity in Colocasia tubers and may interfere with bioavailability of other nutrients. Hence, the reduction of calcium oxalate content determined in the present work is an important contribution to Amazonian and vegan diets, which have a high consumption of vegetables.

3.4 Functional properties of the starches

Water-binding capacity, pH and gel clarity

The functional properties of the isolated starches from X. sagittifolium and P. tuberosus were evaluated according to its water-binding capacity, pH, and gel clarity. The capacity and intensity of water absorption within the starch granules are described as water-binding capacity (WBC) (Deepika et al., 2013).

WBC is associated to the formation of hydrogen and covalent bonds between water and the polysaccharides chains in starch (Jiang et al., 2012). PTS presented a higher WBC then XAS. The results of WBC were 192 ± 17 g/100g and 292 ± 18 g/100g for XAS and PTS, respectively. Similar values (188 - 257 g/100g) of WBC in Dioscorea opposita starch treated with different heat temperatures were reported to influence the gelatinization degree (Chen et al., 2017a). Low values of WBC were attributed to the V-type of the crystallinity pattern in starch from Dioscorea alata L. due to compact granules structure (Mao et al., 2018). The pH of a 1% aqueous suspension (w/v) of XAS and PTS were 4.82 ± 0.03 and 3.84 ± 0.01, respectively. Starch gels clarity is a significant quality for foods because of the influence on its viscosity and appearance (Craig et al., 1989). The isolated starch XAS presented lower gel clarity of 28.5 ± 0.3% then the value, 40.8 ± 0.6%, from PTS. A lower percentage of transmittance is related to opaquer gels (Craig et al., 1989). Gelling is related to granule properties, such as amylose content, pH, granules morphology, WBC and its internal arrangement (Deepika et al., 2013). Paste clarity in normal starches varied from 10% (rice) to 88% (potato), and starch from roots like cassava is around 50% (Sánchez et al., 2010). Gels from cereals exhibit lower clarity than those from roots and tubers (Sánchez et al., 2010).

Crystallinity degree

The starches, XAS and PTS, were analyzed by X-ray diffractometry (XRD) to investigate their type of structural arrangement. X-ray diffraction can reveal the structure order and double helices arrangement, thus reflecting the three-dimensional crystallinity of starch samples (Hornung et al., 2017). The crystalline peaks were analyzed in the interval from 2° to 50° (2θ) and the crystallinity degree was calculated as the ratio of the crystalline diffraction area to the total diffraction area (Huang et al., 2016). The XRD patterns of XAS and PTS, in Figure 3, show that the starches presented reflections intensities at 15°, 17°, 18°, 20° and 23° at 2θ angles, which is typical in an A-type starch structure (Lawal et al., 2008; Zhu, 2016). This result is in accordance with the XRD patterns found in Dioscorea opposita flours and in Xanthosoma sagittifolium native starches (Chen et al. 2017b; Perez-Rea et al., 2013). Crystal type of starch granules possess a closest relation to the amylopectin chains length, in which A, B and C-type crystallinity are linked to short, long and medium chain lengths (Cheetham & Tao, 1998). Relative crystallinities of rice starches were inversely associated to the content of apparent amylose (Chung et al. 2011). Other authors reported an amylose content in Pachyrhizus ahipa and P. erosus ranging from 11 to 23% (Forsyth et al., 2002). The CrD of XAS was, near to 16.6%, a higher value than PTS, around 10.0%. These

Table 2. Calcium oxalate content (mg/100g) in edible tubers.

| Sample               | Uncooked          | Cooked           |
|----------------------|-------------------|------------------|
| Xanthosoma sagittifolium | 699.27 ± 0.1<sup>a</sup><sup>A</sup> | 78.51 ± 1.9<sup>a</sup><sup>A</sup> |
| Pachyrhizus tuberosus  | 499.67 ± 0.1<sup>b</sup><sup>B</sup> | 60.66 ± 0.9<sup>b</sup><sup>B</sup> |

Results are expressed as mean ± standard deviation (n = 3). Lowercase letters <sup>ab</sup> overwritten in the same line shows significant difference (p < 0.05) by T-test. Uppercase letters <sup>AB</sup> overwritten in the same column shows significant difference (p < 0.05) by Tukey’s test.

Figure 2. SEM observations of (a) Xanthosoma sagittifolium and (b) Pachyrhizus tuberosus starches microstructure within a 1000x magnification.
results are lower than the values, 45%, found in native starches from *Xanthosoma sagittifolium* and 34.3% from *Pachyrhizus erosus* (Gunaratne & Hoover, 2002; Stevenson et al., 2007).

**Fourier transform infrared spectroscopy**

The infrared spectrometry (FTIR) is an important tool that supply information about chemical groups and their vibrational state associated to changes in the chemical composition of raw materials (García-Salcedo et al., 2018). The FTIR spectra are shown in Figure 4. The regions near 3307 and 3308 cm⁻¹ are assigned to –OH stretching (Londoño-Restrepo et al., 2018). The bands between 2980-2860 cm⁻¹ are related to vibrational stretch of C–H (Londoño-Restrepo et al., 2018). Bands around 1650 cm⁻¹ are associated to stretching vibration of C=O (amide I) and representing water molecules in the starch amorphous region (Dankar et al., 2018; Kizil et al., 2002; Dlsson & Salmén, 2004). The band near 1420 cm⁻¹ is associated to −CH₂ bending and −C=O stretch (Cael et al., 1975; Dankar et al., 2018). Vibrations of glucosidic bond (C–O–C) are attributed to the band at 1160 cm⁻¹ (Olsson & Salmén, 2004). Higher intensity bands located at ~1120 cm⁻¹ are associated to C–O and C–C stretching vibrations of polysaccharide molecules and have been link to the degree of the hydration of the sample (Cael et al., 1975; Contreras-Jiménez et al., 2019).

**Thermal degradation**

Figure 5 shows the thermogravimetric (TGA) and differential analyses (DTA) of the decomposition behavior of XAS and PTS. Both samples were heated from 25 °C to 700 °C and their thermal degradation process was analyzed through the weight loss (%) and rate of weight loss (%/°C). As observed in Figure 5a, the first mass loss on XAS, ~11%, can be related to moisture content on starch and occurs until ~150 °C. The initial degradation temperature (T_onset) takes place at ~293 °C and the peak temperature at ~310 °C. This second stage of mass loss is associated to main decomposition of the macromolecules on starch. Total mass loss detected at 700 °C was of 88.1%, remaining inorganic ashes after the organic matter carbonization. Similar degradation behavior was described in *X. robustum* starch (Londoño-Restrepo et al., 2014). Figure 5b shows the thermal degradation behavior of PTS. It also noted an initial mass loss, closely to 10% of weight, which occurs until 150 °C and can be correlated to moisture content. T_onset and peak temperature of PTS occurred at ~291 °C and 309 °C, respectively. Total mass loss was of almost 90% at 700 °C. Initial thermal degradation at 220 °C for anhydrous potato starch is describe in the literature (Monnier et al., 2017).

![Figure 3](image1.png)

**Figure 3.** XRD patterns of XAS (black line) and PTS (gray line).

![Figure 4](image2.png)

**Figure 4.** FTIR spectra of XAS (black line) and PTS (gray line).

![Figure 5](image3.png)

**Figure 5.** TGA (black line) and DTA (gray dash line) curves of XAS (a) and PTS (b).
4 Conclusions

The two species of wild tubers studied in this work presented a good physico-chemical composition based on their major amount of carbohydrates, low fats content and relevant amount of crude protein. These tubers were cooked into boiling water to verify the reduction of calcium oxalate content after the application of the thermal processing. A significant reduction of more than 85% in calcium oxalate content was found for both species after cooking. The functional properties and physico-chemical characteristics of starches from these species were also investigated. The isolated starches presented lower gel clarity and a slightly high water-binding capacity, which may be related to the internal arrangement and morphology of the starch granules. A-type starch structure was found in the isolated starches from Xanthosoma sagittifolium and Pachyrhizus tuberosus. XAS and PTS starches presented low values of crystallinity degree, which may be an indication of higher amounts of amylase in it. High intensity bands related to vibrations of glucosidic bonds and others functional groups of polysaccharides were observed by infrared spectrum in XAS and PTS. The thermal behavior analysis of the starches revealed initial degradation temperatures ranging around ~290 °C and peak temperatures close to ~310 °C. Thermal performance of starches generally occurs in similar way due to the degradation of macromolecules in this temperature range. Thus, Xanthosoma sagittifolium and Pachyrhizus tuberosus tubers presented good characteristics to be consumed as a cooked vegetable or as a potential ingredient in food products based on the properties of their starches. Furthermore, the identification of such important properties of these traditional foods of Amazonian communities contributes to increase the concern about conservation of indigenous knowledge and the rain-forest protection.

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