Research Article

Accumulation dynamics and physicochemical variability of starch in cultivars of *Canna edulis* Ker[1]

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ABSTRACT

Canna edulis, also known as achira or sagu, is widely grown in South America because its rhizomes are a major source of starch for food and agroindustry. This study aimed to assess the accumulation rate and physicochemical changes of starch from four canna cultivars (Verde, Nativa, Maituna and Morada), grown under a traditional system, in the southwest region of Colombia. The rhizomes were harvested after planting (between five and nine months) to extract and characterize the starch. It was found that the starch yield (% wb) was related to the plant development age, reaching a maximum at eight months for all cultivars (12.78 ± 0.19 % - Verde; 12.46 ± 0.18 % - Maituna; 12.17 ± 0.19 % - Nativa; 11.10 ± 0.18 % - Morada). The average chemical composition (% wb) of the native starch throughout the rhizome development, for all cultivars, was: 86.68 % of starch, 1.12 % of protein, 0.43 % of dietary fiber, 0.14 % of ash and 11.57 % of moisture. At the optimum harvest age, the minimum and maximum amylose contents were 45.63 % (Maituna) and 54.47 % (Verde). The starch granule size among the cultivars showed a normal distribution, with a range of 40 μm to 80 μm and mean of 59.9 μm. The pasting curves per cultivar showed that the starch generated very high viscosity gels, unstable to the heating-cooling cycles, and high retrogradation.

KEYWORDS: Rhizome, agroindustry, flour.

RESUMO

Dinâmica de acumulação e variabilidade físico-química do amido em cultivares de *Canna edulis* Ker

Canna edulis, também conhecido como achira ou sagu, é amplamente cultivado na América do Sul porque seus rizomas são boa fonte de amido para alimentos e agroindústria. Objetivou-se avaliar a taxa de acúmulo e as alterações físico-químicas do amido proveniente de quatro cultivares de canna (Verde, Nativa, Maituna e Morada), cultivadas sob sistema tradicional, na região sudoeste da Colômbia. Os rizomas foram colhidos após o plantio (entre cinco e nove meses) para extrair e caracterizar o amido. Verificou-se que o rendimento de amido (% bu) estava relacionado à idade de desenvolvimento da planta, atingindo o máximo aos oito meses para todas as cultivares (12,78 ± 0,19 % - Verde; 12,46 ± 0,18 % - Maituna; 12,17 ± 0,19 % - Nativa; 11,10 ± 0,18 % - Morada). A composição química média (% bu) do amido nativo, ao longo do desenvolvimento do rizoma, para todas as cultivares, foi: 86,68 % de amido, 1,12 % de proteína, 0,43 % de fibra alimentar, 0,14 % de cinza e 11,57 % de umidade. Na idade ideal de colheita, os teores mínimo e máximo de amilose foram de 45,63 % (Maituna) e 54,47 % (Verde). O tamanho dos grânulos de amido entre as cultivares mostrou distribuição normal, com amplitude de 40 μm a 80 μm e média de 59,9 μm. As curvas de viscosidade por cultivar mostraram que o amido gerava géis de viscosidade muito alta, instáveis aos ciclos de aquecimento-resfriamento, e alta retrogradação.

PALAVRAS-CHAVE: Rizoma, agroindustria, farinha.

INTRODUCTION

Throughout history, starch has been a fundamental food worldwide. It is found in all plant tissues, but it is most abundant in storage organelles called amyloplasts, which are present in seeds, tubers, roots and legumes (Šárka & Dvořáček 2017). In corn and potato, each amyloplast contains a single starch granule called simple granule. When two or more granules occur in a single amyloplast, this is called composite granule, as found in rice, oat, cassava root, sweet potato (*Ipomoea batatas* L.), sago (*Metroxylon* sp.) and dasheen (*Colocasia esculenta*) (Bemiller & Whistler 2009).

Canna edulis Ker, also known as achira or sagu, is a perennial plant from the Cannaceae family, native to the South America Andean region, and cultivated mainly for its corms or rhizomes, which grow parallel to the soil surface. As a decoction, the rhizomes are used as a diuretic, the juice as an

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antiseptic, and the stems and leaves are used as fodder for livestock (Mishra et al. 2012). It is an important species for human consumption and agroindustry. The starch is easily digested and used to make bread, biscuits, cookies and tagliatelle (Huang et al. 2013a, Huang et al. 2013b). In the processing of protein-enriched *C. edulis* starch noodles, the native starch is pre-gelatinized by drum drying and, ultimately, noodles with the best cooking stability are obtained by mixing 12 % of pre-gelatinized starch, 28 % of native starch and 60 % of water (Xie et al. 2019). This crop is currently harvested on a family and semi-commercial scale for starch production in small factories in Colombia, Brazil, China, Taiwan and Vietnam (Sjöö & Nilsson 2018).

A study of the phenolic compounds and their antioxidant activity in *C. edulis* residue identified a new molecule [4-(3-(3,4-dihydroxyphenyl)acryloyl)-6-hydroxy-1-methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid] in the water-soluble extract, with potential as a natural food additive (Zhang et al. 2011a). The *C. edulis* species is also a potential feedstock for ethanol production, because of its low nutrition requirements and the high starch content of its rhizomes. Reduction of the biomass viscosity by cell wall-degrading enzymes, including acid xylanase and β-glucanase, achieved 98.3 % of mass fraction of the theoretical ethanol yield in a 5-L fermenter (Huang et al. 2013c).

The *in vitro* fermentabilities of raw and cooked canna starches and their derivatives showed that raw native canna starch generated butyric acid as a main short-chain fatty acid, while acetic acid was a major short-chain fatty acid derived from raw modified starches, highlighting this raw material as a potential source of these types of organic acid (Wandee et al. 2017). The processing of canna rhizomes to extract starch produces vast quantities of byproducts, composed mostly of cellulose and pectic substances. This soluble dietary fiber can be further used as a dietary supplement and additive in the food industry (Zhang & Wang 2013).

Starch from *Canna* species presents opportunities to be used in food and pharmaceutical industries. Research that supports these applications recommends improving the production, extraction methods and starch quality (Andrade-Mahecha et al. 2015). It is important to remember that starches provide functional properties, both in manufacturing processes and final products. These properties are directly related to the chemical components and their physical structures, which depend mainly on their source of origin (Wandee et al. 2015).

This research aimed to identify the starch accumulation dynamics in rhizomes from four *C. edulis* cultivars produced in Huila (Colombia) and how the physicochemical properties change with the plant development, thereby contributing to improve the crop production system and to identify possible competitive advantages of this starch in the agroindustrial sector.

MATERIAL AND METHODS

Plants of *C. edullis* were cultivated at 1,700 m above the sea level in Palermo, Huila, Colombia, between January and September 2018, under an average temperature of 18 ºC and rainfall of 1,200 mm.

Experimental sowing occurred in a randomized complete block design, with three blocks, each with four cultivars (Verde, Nativa, Maituna and Morada) and five replicates per cultivar. The plots consisted of five plants, for a total of 300 plants in the experiment. The land was leveled and weeded. Holes of 15 cm × 15 cm and 30-cm depth were opened at inter-hole distances of 1.0 m × 1.0 m, and filled with organic fertilizer (500 g). Weed control occurred manually every 15 days. According to technical recommendations, chemical fertilization and irrigation are unnecessary (Thitipraphunkul et al. 2003a).

The rhizomes were harvested every 30 days, from five to nine months. Harvesting involved manually tearing the plants with a shovel, shaking to loosen soil, cutting the stem base to separate rhizomes, and removing dry material, to finally pack and transport the plants to the processing site. Each sample (rhizomes of five plants per plot) was analyzed independently. Yield was evaluated based on the raw starch content. Laboratory-scale extraction was carried out based on a traditional procedure (Wandee et al. 2015).

Native starch from all cultivars was analyzed for moisture (AOAC 960.52), ash (AOAC 960.52), protein (AOAC 920.87 and using 6.25 as the crude protein factor) and dietary fiber, which was quantified by adopting a standard protocol (AOAC 985.29), using a TF-100 kit (Sigma-Aldrich). The starch content was estimated by the amylose/amyloglucosidase method, using an STA-20 kit (Sigma-Aldrich). The

apparent amylose content was calculated based on the iodine-amylose complex formation (ISO 6647-1:2015), measured at 620 nm wavelength, using a spectrophotometer (Helios Zeta, Thermo Scientific), and quartz cuvette with 10 mm path length. The data were compared with an amylose/amylopectin standard curve (0 % to 70 % of amylose) that had a quadratic correlation coefficient (r^2) of 99.3 %.

Granule size was measured using an optical microscope (Nikon Labophot-Pol) coupled with a 20-MP digital camera. Two starch suspensions per sample (0.1 % w/v) were shaken for 5 min, and then stained with an I_2/KI solution (0.2 %). Before the observations at ×40 magnification, a calibration was performed using a micrometric slide (Carl Zeiss) with 10-μm divisions, determining that forty divisions of the eyepiece were equal to 204.88 μm. Then, the following equation was used to transform the observed starch granule diameter (A, μm) in respective size (S, μm): S = (A × 204.88)/40.

In equiaxed granules, size was expressed as diameter, while, in non-equiaxed granules, size corresponded to the longest distance between granule edges. Measurements were made on a sample of 200 granules using the ImageJ® software (NIH, USA), and size distributions were carried out using the Statgraphics Centurion XV® - PStatPoint Technologies (Perez 2002). In order to determine the pasting curves, starch suspensions were prepared at 10 % w/w (10 g of starch in 100 mL of water). The mass of added water was corrected to a 12 % moisture content basis. These solutions were transferred to a

Micro Visco-Amylo-Graph® (Brabender), where they were heated up to 95 ºC at 6 ºC min⁻¹, held at 95 ºC for 3 min and then cooled to 50 ºC at the same rate, and held for 1 min. The following variables were obtained from the generated curve: temperature at which the gel formation started (ºC), maximum temperature (ºC), maximum viscosity (Brabender units - BU), minimum viscosity (BU), final viscosity (BU), breakdown (BU) and setback (BU).

The data were statistically adjusted by analysis of variance and means comparison (LSD) test (at 5 % of significance) to verify if there were significant differences in the starch contents among the different growth stages for each cultivar. Correlation analyzes were also performed to identify the mathematical models that best described the change in physicochemical characteristics. Theses analyses were also performed using the Statgraphics software (Perez 2002).

RESULTS AND DISCUSSION

The starch accumulation dynamics in *C. edulis* rhizomes for each evaluated cultivar (Figure 1) revealed an increasing tendency of average starch yield (% wb) as the physiological age advanced, but only until eight months. At this point, the starch content decreased. Significant differences ($p < 0.05$) in the starch yields between cultivars were noted from the first sampling (five months). The average rate of starch accumulation between five and eight months was 1.55 % to 2.11 % (wb) by month, with
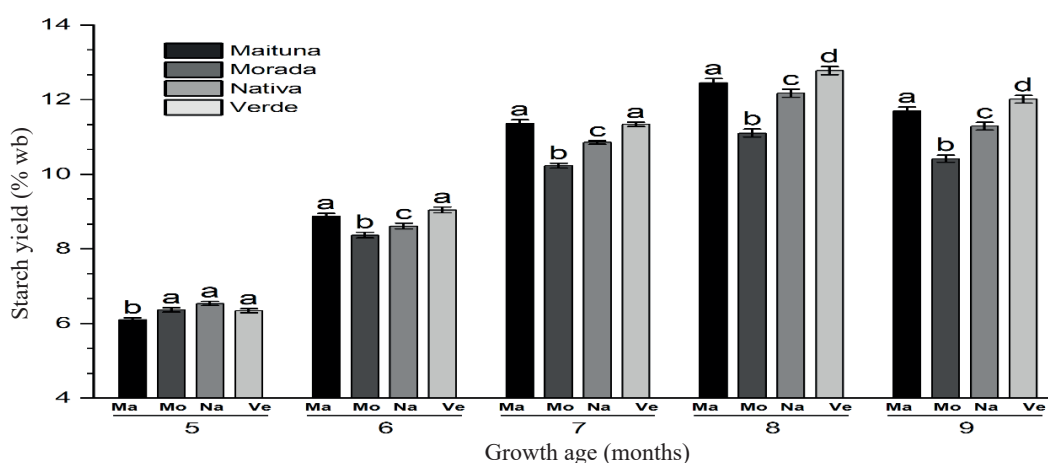

Figure 1. Starch yields variation in four *Canna edulis* cultivars at different vegetative growth ages (bars above each column represent standard errors of the means, which, if followed by the same letter for each month, do not differ in *post hoc* test at 5 % of probability).

the Morada and Verde cultivars representing the minimum and maximum, respectively.

To obtain the highest starch concentrations, the appropriate harvest time for all studied cultivars was eight months. With significant differences in the starch contents among cultivars, a multiple range analysis (LSD) was carried out obtaining three homogeneous groups (% starch wb): Morada 11.10 ± 0.18 (group 1); Maituna 12.46 ± 0.18 and Nativa 12.17 ± 0.19 (group 2); Verde 12.78 ± 0.19 (group 3). This behavior may be affected by the climatic conditions in which the plant grows - total solar radiation, photoperiod and relative humidity (Fonseca-Florido et al. 2016).

The Verde cultivar stood out for its greater precocity and the largest number of rhizomes (between 45 and 60), which were ovoid and between 8 cm and 12 cm long, while Nativa presented a lower number of rhizomes, but with a larger size (10-20 cm). At the last sampling (nine months), an approximate 1 % (wb) decrease in the starch yields was observed for all cultivars because, once the plant reaches its maximum leaf number, it starts consuming its reserves to sustain its aerial development (Pagán-Jiménez et al. 2015).

From the proximal analysis data of all starch samples (Figure 2), the moisture content range was 10.55-13.49 %, which is adequate for this product. Under ambient temperature, commercial native starches contain around 12-17 % of moisture, as a low water content favors their preservation and storage (Andrade-Mahecha et al. 2012). From the extracted raw starch, the net starch percentage increased slightly with the rhizome development, varying from 1.74 % (Morada) to 3.64 % (Nativa), with intermediate values of 2.13 % (Verde) and 2.64 % (Maituna). These data suggest that the net amount of starch does not change significantly after five months. This is consistent with the fact that the increase in rhizome size is the factor that most influences the raw starch yield.

The starch extracted from the cultivars at different physiological ages (months) had comparable protein amounts (0.72-0.95 % wb), which were higher than reported by Zhang & Wang (2013) in *C. edulis*, but similar to potato starch (Klaochanpong et al. 2015). The low dietary fiber content, in comparison to other vegetable sources, is attributed to the protein contribution, decreasing the dietary fiber content by 0.20 % (Verde), 0.22 % (Nativa), 0.29 % (Morada)
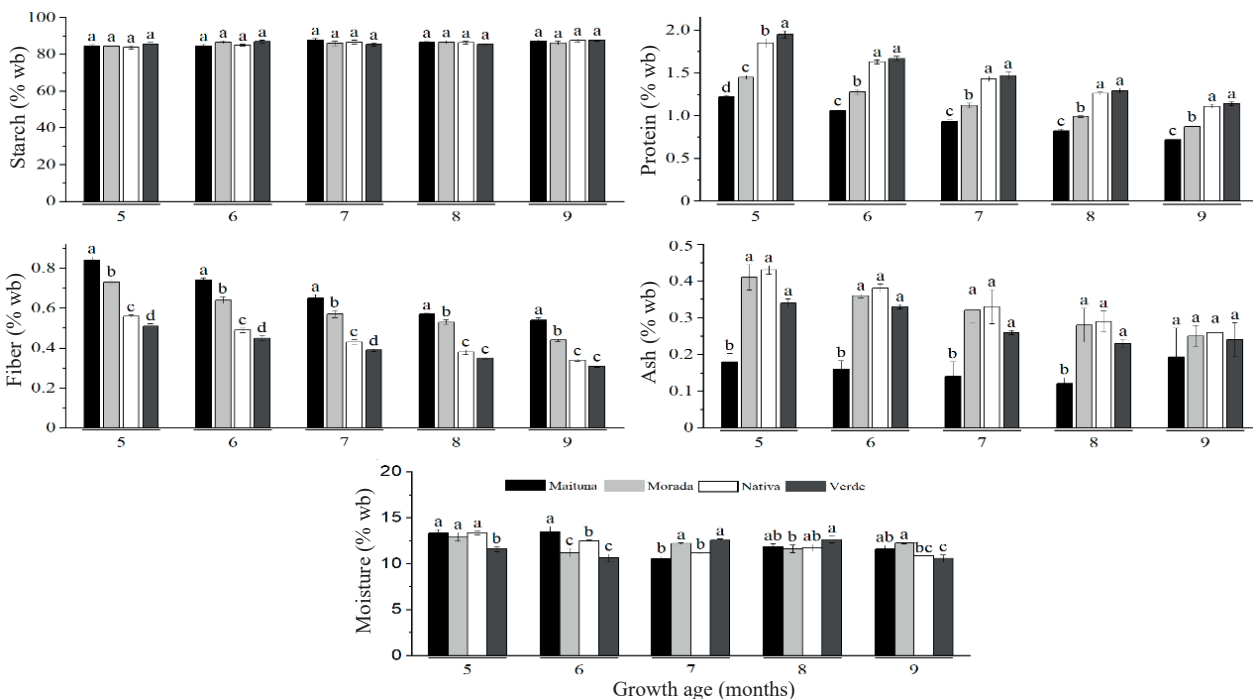

Figure 2. Native starch proximal composition (% wb) in four *Canna edulis* cultivars at different vegetative growth ages (bars above each column represent standard errors of the means, which, if followed by the same letter for each month, do not differ in *post hoc* test at 5 % of probability).

and 0.50 % (Maituna). The ash contents were within the range expected (0.03-0.26 %) from the literature data (0.18-0.33 %) (Puncha-Arnon et al. 2007).

In all cultivars, the starch exhibited a high amylose percentage, with averages of 43.09 % as a minimum (five months) and 53.55 % as a maximum (nine months). There was an increasing relationship between the amylose content and rhizome age (Figure 3), observed in all four cultivars. In contrast, amylopectin decreased progressively with the plant development, what corroborates another research (Lan et al. 2015). In other studies about canna starch structure (Thitipraphunkul et al. 2003b, Cisneros et al. 2009), amylose was categorized as medium-sized (1,500-1,600 degrees of polymerization, DPn), when compared with that in starch from other sources, such as potato (4,370 DPn), sweet potato (3,230 DPn), barley (1,580 DPn), wheat (1,220 DPn) and maize (1,030 DPn). Starch containing amylose with low polymerization degree produces unstable gels, but the high levels of such amylose present in *C. edulis* starch may be beneficial in improving film formation properties, as reported in the two referred studies.

The Verde cultivar recorded the highest amylose contents throughout the analyzed period, while the Maituna ecotype always showed the lowest amylose values, with a maximum of 46.97 % at nine months. The amylose-to-amylopectin ratio of starch greatly affects the starch functionality, including a gelatinization behavior and the final gel characteristics (Zhang & Wang 2012).

There is often a positive correlation between amylose content and granule size, as found in the current analysis. However, this trend is not absolute, because many interacting factors influence the starch granule architecture and composition. It is expected that the gelatinization of starch with high amylose content, such as observed for *C. edulis*, will form a firm and cuttable gel. On the other hand, starch high in amylopectin will present a high viscosity peak, followed by a break during heating, without gel formation (Fonseca-Florido et al. 2018).

Besides the amylose and amylopectin contents, differences in the pasting properties may be attributed to the lipid content and the distribution of the amylopectin branches (in canna starch, it should be
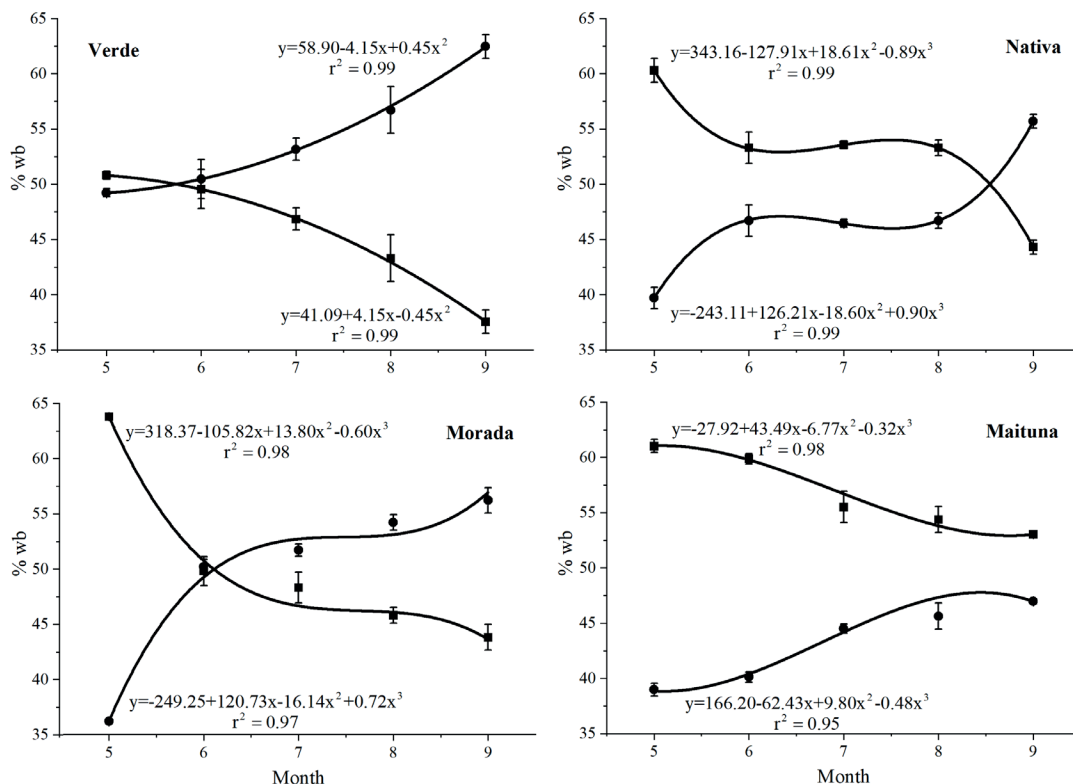

Figure 3. Contents (% wb) of amylose (●) and amylopectin (■) in the starch from four *Canna edulis* cultivars at different vegetative growth ages, in months (bars for each observed point represent the respective mean standard errors).

remembered that amylopectin branches are longer than those present in cassava and bean starches), as long chains of amylopectin interact more easily with amylose, generating starch gels with more viscosity (Fuentes et al. 2019). In general, functional starch characteristics are determined not only by the whole granule properties, but also by its amylose and amylopectin components (Dhital et al. 2019).

Microscopic observations allow to identify differences in granule forms and appearances among starches. In the current study, the granules exhibited a smooth surface, oval and round shapes, with few broken granules, indicating low levels of starch damage during the extraction (Fonseca-Florido et al. 2017). All cultivars presented an average granule size between 38.14 μm and 46.88 μm for the first five to seven months of physiological age. The size distribution profiles for the optimum harvest time (eight months) are showed in Figures 4 and 5. It demonstrates a predominance of sizes between 40 μm and 80 μm, with an average of 59.9 μm, which classifies it as a large-granule starch, in agreement with a previous study by Zhang et al. (2014).

These granule sizes in *C. edulis* suggest some possible applications. For example, granules smaller than 60 μm are used in formulating cosmetic products because of their high-water absorption capacity, and as an encapsulating agent for flavors and dyes. The starch granule size is also an influential factor of starch digestibility and stability at high temperatures (Zhang et al. 2011b). The large size of the granules in canna starch confers it useful properties in the manufacture of traditional "Achiras" biscuits in the region of Huila (Colombia). Canna starch is used in the Asian cooking to make pasta, as well as a
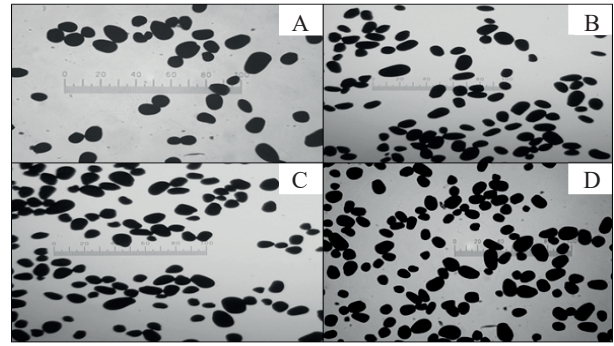

Figure 5. Starch granules micrographs (objective: ×40) from four *Canna edulis* cultivars (A: Maituna; B: Morada; C: Nativa; D: Verde) for harvest at eight months.

thickener in soups and sauces, and, when modified, it has been used to encapsulate food ingredients, such as flavors (Andrade-Mahecha et al. 2012, Gómez-Aldapa et al. 2019).

It was evidenced here that *C. edulis* starch granules are larger in relation to other sources, such as corn, cassava and potato, in which the average granule size is 12 μm, 10 μm and 48 μm, respectively (Puncha-Arnon et al. 2008). It has been proposed that larger granules gelatinize first and the smaller ones later, although this is not a universal standard, and smaller granules have a greater solubility, swelling power and water absorption capacity than larger granules (Zhang & Wang 2012, Zhang & Wang 2013).

The gelatinization process is defined as the collapse or disruption at the molecular level within the starch granule, manifesting irreversible changes in properties such as granule swelling, crystalline structure fusion, birefringence loss and solubilization
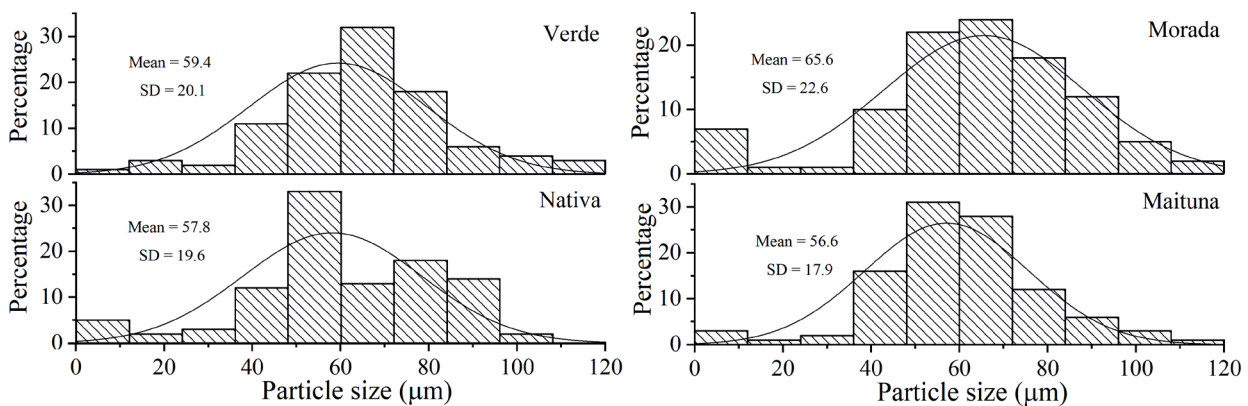

Figure 4. Size distribution of starch granules (μm) in four *Canna edulis* cultivars for harvest at eight months. SD: standard deviation.

(Pulido Díaz et al. 2017). The visco-amylo-graph profiles are shown in Figure 6. All samples presented approximately a similar behavior, including a high viscosity, instability throughout heating at 95 ºC and a moderate setback, although discrepancies were observed in the pasting variables. These differences in the gelatinization starting point and range over which it occurs might be a result of the starch concentration, observation method, granule type and granule population heterogeneity (Dewi 2009).

The starch gelatinization starting point was 63.6-66.6 ºC, and the highest gelatinization temperature was similar among all cultivars, with a difference of 1.8 ºC between the maximum and minimum found. According to Grunina et al. (2015), this variation in the starch samples may be caused by differences in cropping practices, ecotypes or environmental conditions in which the plant grows.

Table 1 shows the changes in pasting parameters across the thermal process. The maximum viscosities

were between 924 BU and 1,210 BU, differences that may be attributed to the amylose and amylopectin chains distribution and amylose content (Huang et al. 2015). Starches of a relatively larger granule size (Verde and Morada cultivars) recorded the highest maximum viscosity. For all pastes, viscosity decreased considerably after heating at 95 ºC, with Verde and Morada displaying the highest breakdown of 782 BU and 735 BU, respectively, indicating a high instability. This starch behavior depends mainly on the amylose content and structural conformation. In processed foods, this phenomenon is considered very important, because of its association with properties such as stickiness, water absorption and digestibility (Jiranuntakul et al. 2011).

After cooling at 50 ºC, the final viscosity of all starches increased moderately, resulting in a minimum setback for Nativa (126 BU) and a maximum one for Morada (178 BU). After cooling the *C. edulis* starch, there is less available energy
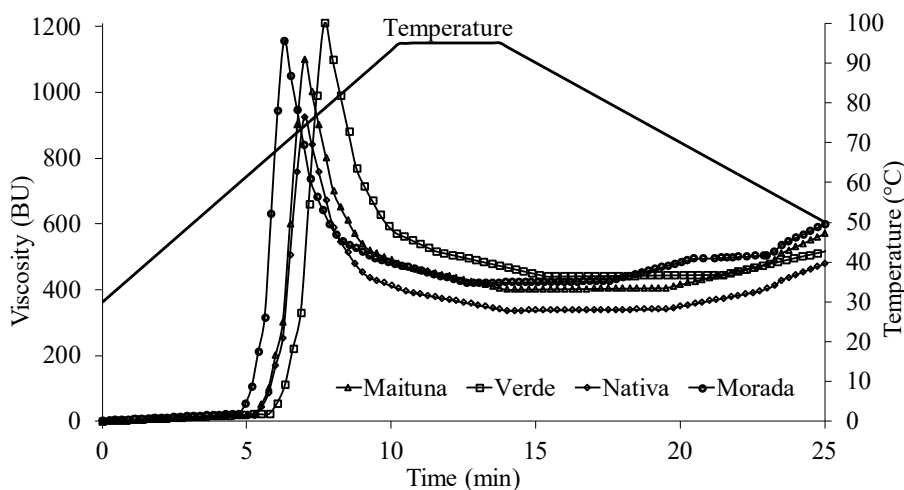

Figure 6. Starch visco-amylo-graphs from four *Canna edulis* cultivars, as a function of the temperature, for harvest at eight months. BU: Brabender units.

Table 1. Starch pasting properties (mean ± standard error) from four *Canna edulis* cultivars, for harvest at eight months.

Properties	Cultivars			
	Verde	Nativa	Maituna	Morada
Gelatinization starting point (ºC)	65.5 ± 1.3	66.6 ± 0.7	63.6 ± 2.4	66.5 ± 1.8
Maximum temperature (ºC)	71.2 ± 1.2	69.6 ± 1.3	69.4 ± 1.2	70.7 ± 1.1
Maximum viscosity (BU)	1,210 ± 13.7	924 ± 10.8	1,100 ± 46.7	1,155 ± 13.7
Minimum viscosity (BU)	440 ± 14.4	336 ± 7.2	400 ± 7.6	420 ± 12.4
Final viscosity (BU)	517 ± 20.2	462 ± 18.6	570 ± 20.8	598 ± 15.5
Breakdown (BU)	782 ± 24.6	558 ± 0.8	700 ± 37.2	735 ± 18.4
Setback (BU)	150 ± 6.1	126 ± 6.5	170 ± 1.9	178 ± 2.7

BU: Brabender units.

to keep the solubilized starch molecules separated, because of the high amylose content, leading to crystalline aggregates and gel texture. Over time, this gel becomes gummy and tends to release water (syneresis), a phenomenon common in products such as sauces that have been formulated with amylose. The opposite trend is presented with starch of high amylopectin content, because it improves the texture and homogeneity of gelled starch, promoting starch gel stability when freezing and defrosting foods formulated with high-amylopectin starch (Puncha-Arnon et al. 2015). It must be remembered that Andean starches from rhizomes such as *C. edulis* exhibit higher viscosity values, when compared with corn (700 BU), wheat (250 BU) and rice (500 BU), but lower than for potato (2,900 BU) and tapioca (1,200 BU) (Dhital et al. 2019).

CONCLUSION

The starch accumulation rate in *Canna edulis* rhizomes varied with the plant development, revealing that eight months is the optimum harvest point for the conditions and vegetal material studied. The Verde and Maituna cultivars registered the highest yields in raw starch. The physicochemical composition was similar among all the cultivars, highlighted by a high amylose content, and a high percentage of large starch granules was evident from the size distribution analysis. The pasting behavior showed a marked tendency to generate high viscosity gels, unstable to the heating-cooling process, high retrogradation, and a moderate recovery of the final viscosity.

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