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Physicochemical and digestibility characteristics of starch isolated from Andean tubers produced in Colombia

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Physicochemical and digestibility characteristics of starch isolated from Andean tubers produced in Colombia

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Abstract

The physicochemical characterization of non-conventional starches from the Andean region of Colombia was carried out, on Arracacha (*Arracacia xanthorrhiza*), Hibia (*Oxalis tuberosa*), Chugua (*Ullucus tuberosus*), and Cubio (*Tropaeolum tuberosum*). This study investigates the physicochemical properties of starches derived from Andean tubers, focusing on starch purity, amylose content, molecular weight, chain length distribution, short-range order, thermal properties, morphology, and digestibility, both in their raw and cooked forms. Tests were conducted in duplicate or triplicate, depending on the specific technique and solution availability, followed by statistical analysis of the results. Arracacha exhibited the highest starch purity at 90.3%. The amylose content ranged from 17% to 25.51%, indicating significant functional variability among starches. Hibia and cubio starches demonstrated the highest short-range order, with ratios of 1045/1022 and 1022/995, respectively. Chugua starch exhibited the highest amylose molecular weight (2.51 x10^6 g/mol), while hibia starch had the highest amylopectin molecular weight (2.85 $x10^6$ g/mol). Morphological analysis revealed distinct differences, with hibia and chugua starch granules appearing elongated, while arracacha and cubio granules were polyform and spherical. Gelatinized starches showed low digestion rates (less than 40%), with resistant starch content ranging from 43.89 % to 46.5%. These findings highlight the nutritional and functional value of Andean tuber starches, presenting promising alternatives for the food industry.

Keywords: Andean tubers; thermal properties; kinetic digestibility.

1. Introduction

Recent trends in food consumption have shifted towards gluten-free products and a reduction in the intake of ultra-processed foods (UPF). Food items such as bread, spaghetti, ready-to-eat cereals, soups, and fries are currently under scrutiny, as they fall within the category of UPF. Moreover, these products typically contain wheat flour and isolated starches derived from conventional botanical sources like corn, potato, and wheat. These ingredients are associated with rapid digestible starch content and are implicated in the prevalence of obesity. Consequently, there has been a concerted effort within the scientific community, to explore non-conventional sources of starch. These alternative sources are envisioned to possess functional attributes akin to traditional starches while concurrently exhibiting diminished digestibility. Such novel starch sources hold promise for application in gluten-free and UPF-free food formulations.

In South America, a diverse array of root and tuber crops are cultivated, playing a fundamental role in the region's culinary landscape. Recent investigations into starch isolated from tubers including *Oxalis tuberosa*, *Tropaeolum tuberosum*, and *Ollucus tuberosus* have unveiled a significant presence of resistant starch, underscoring its potential utility in the development of innovative functional food products (Velásquez-Barreto & Bello-Pérez, 2023). Daza et al. (2022) documented the digestibility fractions of *Tropaeolum tuberosum* grown in Colombia, revealing a remarkable resistant starch content of 94.4 %. However, Velásquez-Barreto et al. (2021) investigated the digestibility of starches derived from *Oxalis tuberosa*, Tropaeolum tuberosum, and *Ollucus tuberosu*s, cultivated un Perú, in both their raw and gelatinized states. Their findings revealed a substantial range of resistant starch content in the raw starches, spanning from 58.47% to 77.34%. Furthermore, upon analysis of the gelatinized starches, a notable decrease in the resistant starch fraction was observed, ranging between 21.62% and 26.41%. Additionally, the slow-digesting fraction was reported to range from 2.66% to 9.93%. The variable results in resistant starch contents can be attributed to structural changes in the starches, highlighting the need for further research on digestibility and structural alterations according to the geographical regions where the product is cultivated. Sanchez-Portillo et al. (2023) published a review about morphological diversity and agronomic management of Anden Tubers. Those authors concluded that the spectrum of Andean tubers exhibits substantial disparities in their nutritional profiles, morphological attributes, and indeed, the physicochemical properties of starch.

The chemical composition of starch is characterized by two carbon chains, amylopectin, and amylose. Starch, extracted from various sources, displays distinct properties determined primarily by the ratio of amylopectin to amylose These variations impact solubility, viscosity, strength, and other characteristics. These concentrations are influenced by the biotic source from which the starch is derived. Given the diverse range of available sources, starch exhibits potential applications beyond nutrition (Apriyanto et al., 2022). Despite the existence of materials with similar attributes, starch remains widely utilized due to its abundance and the diversity of sources available for extraction. Consequently, numerous scientific disciplines and engineering fields leverage starch for research, development, and the innovation of novel products (Rodrigues et al., 2021).

Starch finds utility in a myriad of industries such as cosmetics, baking, and textiles, where it either substitutes raw materials or serves as a foundational component for manufacturing processes. Its versatility makes it indispensable for applications requiring adaptability and pliability. Although starch may not enjoy ubiquitous usage across all scientific domains, notable advancements have been achieved in those where it is employed (Mary et al., 2022).

A research stay was conducted to study the physicochemical characteristics and digestibility of isolated starch from four tubers and rhizomes grown in Colombia. Starch isolated from Arracacha (*Arracacia xanthorrhiza*), Hibio (*Oxalis tuberosa*), Chugua (*Ollucus tuberosus*), and Cubio (*Tropaeolum tuberosum*) were analyzed in this study. This stay took place between January and February 2024 at the Center for Bioproducts Development of the National Polytechnic Institute of Mexico (IPN) with Dr. Luis Arturo Bello Pérez.

2. Materials and methods

2.1. Sample Selection and Starch isolation.

Tubers that are unconventional or less commonly used in these studies were selected for starch extraction. These tubers were sourced from the Cundí Boyacense high plateau region of Colombia, characterized by mostly cold climates and situated well above sea level. These conditions favor the cultivation of various tubers throughout the year, as climatic conditions remain stable. In this case, tubers commonly used in a traditional Colombian dish called "*cocido boyacense*" were selected. Arracacha (*Arracacia xanthorrhiza*), Hibiscus (*Oxalis tuberosa*), Chugua (*Ollucus tuberosus*), and Cubio (*Tropaeolum tuberosum*). The tubers were purchased in a local market at Bogota.

The starch extraction was carried out by wet milling process. Briefly, 3 kg of fresh tubers were weighed, washed, chopped, and separated accordingly. Subsequently, the sample was suspended in sodium metabisulphite (1 %) in a relation of 1.2 solid.liquid. The sample was mixed thoroughly,

and then blended for approximately 1 min. Each sample was then passed through 50, 100, and 200 U.S. sieves. Once the sample passed through the final sieve, it was allowed to settle, and the supernatant was decanted, leaving only the precipitate. This precipitate was placed in aluminum trays and dried in an oven at 45°C for 48 h. Finally, the dried sediment obtained was pulverized, weighed, packaged, and labeled for subsequent use. This process was carried out for each of the 4 samples.

2.2. Moisture Determination

For the moisture determination of the samples, an analytical balance was used to first weigh the aluminum trays in which the samples would be placed and approximately 500 mg of each sample. These trays were then placed in a desiccator and subsequently transferred to a drying oven at 100°C for 5 hours. After the designated time, the samples were transferred to a desiccator and weighed individually, with no more than 20 min elapsing between removal from the oven and weighing. This process was carried out in triplicate for all 4 samples. Finally, a formula was applied using Excel to estimate the moisture content of each sample.

2.3. Starch purity

The starch purity was determined following the measure of Total Starch Content, kit from Megazyme International Ireland Ltd., according to item E for samples containing D-glucose and/or maltodextrins and item C for samples that contain resistant starch to flour and starch analysis, respectively (Megazyme International Ireland, 2011b). This method is based on measuring the amount of glucose released after an enzymatic hydrolysis.

2.4. Amylose/amylopectin fraction

For the determination of amylose and amylopectin in the samples, the method proposed by Megazyme for native starches was utilized kit (Megazyme International Ireland, 2011a).

2.5. Short-range organization

The short-range organization of starch granules was measured through attenuated total reflectance total reflectance Fourier Transformed Infrared Spectroscopy (FTIR-ATR) in a Bruker Model Vertex 70 spectrometer (Briker Optics GmbH, Ettlingen, GER). The spectrometer setting was referred from Romero Hernández et al. (2023).

185

186 *2.6. Molecular Weight*

187 Chromatography was employed to determine Average molecular weight (Mw) and gyration radii (Rz)
188 were calculated using ASTRA® Version 5.3.1.5 software (Wyatt Technology Corporation, Santa
189 Barbara, USA), Berry method and second order polynomial were used. For this, 15 mg of each sample
190 was taken, and 1.5 ml of Dimethyl sulfoxide 95% was added. They were then placed in an agitator at
191 80 °C for 24 h. Subsequently, 1 ml of the supernatant from each sample was taken and placed in
192 standard chromatography vials, the AF4 system for chromatography were used.
193 AF4 system consisted in a short channel (Wyatt Technology Corporation, Santa Barbara, USA) with
194 a 10 kDa membrane, coupled to a multiangle laser light scattering-MALLS (Dawn Heleos 8, Wyatt
195 Technology Corporation, Santa Barbara, USA) and refractive index detector-RID (1100 Generic RI,
196 Agilent Technologies, Santa Clara, CA, USA) (Hoyos-Leyva et al., 2017).
197 An injection volume of 1 ml was set at a speed of 100 µl/h, and a time of 90 min per vial was
198 configured and columns of 30 – 3000 Gram were used. This test was conducted in duplicate for each
199 sample, plus a blank. Once the results were obtained, the baselines were defined and integrated, peaks
200 were defined, and the graphs were normalized. After this process, the program generated the desired
201 parameters' results. Once these data were obtained, they were transferred to a specialized Excel sheet
202 where it was possible to obtain noise-free graphs and gyration radius parameters for both amylose
203 and amylopectin.
204 Finally, a debranching process was applied to the starches, with the results and graphs analyzed in
205 Origin for their use in the chain length distribution technique, the test specifications were The
206 debranched starches were analyzed by gel permeation chromatography (GPC) on two-system
207 columns (Superdex 200 pg and Superdex 30 pg) connected in tandem, equipped with a 515 HPLC
208 pump (Waters Corporation, Milford, Massachusetts, USA) and fractions collector Frac 920 (GE
209 Healthcare Bio-Sciences AB, Uppsala, Sweden). The descendent flow was 0.4 mL min1 with sodium
210 acetate (0.1 M, 0.02% sodium aside, pH 4.7) as eluent(Hoyos-Leyva et al., 2017).
211
212
213 *2.7. Chain Length distribution.*
214 For the determination of chain length in the samples, 20 mg of each sample was taken and placed in
215 10 ml test tubes. 1.5 ml of dimethyl sulfoxide was added to each tube, and they were shaken and
216 passed through 0.45 µm filters. Subsequently, they were sealed with paraffin paper and placed in
217 freezing for 2 hours. Afterward, the tubes were placed in a specialized vessel, which was then
218 placed in a lyophilization unit for 24 h until all moisture was removed from the sample. Then, 15

mg of the sample was transferred to chromatography vials, and 1.5 ml of deionized water was added. This procedure was carried out in duplicate for each sample. The vials were placed in the equipment and columns of 10 -1000 Gram were used; an analysis of 30 min was configured for each vial. Once completed, the baseline of the graphs and peaks were manually defined. Using the results of the chromatography technique for unbranched starches, the mass fraction of amylose and amylopectin in each sample was determined. Finally, the data was transferred to Origin, graphed on a logarithmic scale, and the degrees of polymerization of amylose and amylopectin were determined.

2.8. Thermal properties

For the calorimetry study, 2 mg of each sample were measured in specialized trays. Subsequently, 7 µl of deionized water were added to each tray, which were then sealed with a press and placed in the calorimetry equipment (TA Instrument, Q20, New Castle, NJ, USA), first the temperature was equilibrate at 30°C, the heating ramp were 10°C/min to 120°C and equilibrate again at 30°C. The thermal properties measured were as follow. onset gelatinization temperature (To), peak gelatinization temperature (Tp), final gelatinization temperature (Tf), gelatinization enthalpy (ΔH).

2.9. Morphological characteristics

The morphology characteristics of Andean tubers starch granules were observed by optical and polarized light microscopy, and low vacuum scanning electron microscopy (LV-SEM). An appropriate microscope equipped with a camera connected to a computer, allowing for the addition of scales and the capture of Figures of the specimens on the slide, was used. The samples were observed using magnification lenses of x80 and x100, and an Figure of the observed material was captured. A scale was added using software assistance. Subsequently, the process was repeated using polarized light microscopy.

Low Vacuum Scanning Electron Microscopy (LV-SEM) was employed to examine the fine structure of native starches.

2.11. Starch digestibility kinetic

To measure the digestibility of the samples, the kinetic method was employed, which involves in vitro simulation of the digestion process. Initially, Buffer A (pH 6.0, 0.2M), Buffer B of α-amylase (pH 7.0, 14.4 mM), pepsin, and a solution of pancreatin and amyloglucosidase were prepared.

The digestibility experiments were carried out following the methodology proposed by Bello-Perez et al., (2019). Briefly, the simulation of the oral phase of human digestion started with 100 mg of starch-based sample was taken and placed in 25 ml tubes with magnetic stirrers. 4 ml of Buffer A and 1 ml of α-amylase were added. For the gastric phase, 2 ml of pepsin was added, and the tubes were incubated at 37 °C with agitation for 30 min. The tubes were then removed from the bath, and the enzymes were neutralized with 0.2 M NaOH. For the intestinal phase, 4 ml of Buffer A was added, and the tubes were places in the incubator again. 1 ml of the pancreatin and amyloglucosidase solution was added, and the timer was started. Aliquots of 100 µl were taken at time points of 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 240, and 360 min and placed in 1.5 ml Eppendorf tubes containing 300 µl of 0.3 M N2CO3 to stop enzyme activity. The Eppendorf tubes were centrifuged at 2000 rpm for 5 min. Subsequently, 10 µl from each eppendorf tube was added to a multi-scan rack along with 290 µl of GOD-POD (glucose oxidase-peroxidase coupled method), and absorbance was measured at 510 nm. This process was conducted in duplicate for each sample.

A digestibility kinetic was graphicated. From the kinetic graph it was possible to determine the values of resistant starch (RS), rapidly digestible starch (RDS), and slowly digestible starch (SDS), as well as the K constants of the latter two, for each of the samples.

These procedures were repeated, both raw and gelatinized starch. The gelatinized samples were boiled once Buffer A was added for approximately 15 min.

2.12. Statistical analysis

All analyses were conducted in triplicate to ensure accuracy and reliability. Mean values along with their corresponding standard errors (SE) were meticulously reported. Analysis of variance (ANOVA) was utilized to discern statistical disparities ($p < 0.05$), with the Tukey test employed as the mean comparative test at a significance level of 0.05. The statistical computations were performed using JMP version 10.0 software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Moisture Determination in Starches

In Table 1, we can observe that the selected uncommon native starches have around 10% moisture content, which is lower than that of more common starches. Particularly noteworthy is the case of Chugua, as it is a tuber with a high-water content; nevertheless, the moisture content of its starch is relatively low.

Table 1. Moisture content (%) in the starch isolated form different Andean tubers from Colombia.

Sample	Humidity (%)
Arracacha	12.49±0.23
Hibia	9.79±0.47
Chugua	10.97±0.70
Cubio	8.73±0.39

3.2. Determination of Total Starch

When performing the total starch technique, it is possible to observe in Table 2 that the percentages of total starch are relatively low for tubers, both on a dry basis and on a wet basis, where they should be above 90%. This may be due to the starches not being purified before the technique was carried out, which could result in impurities affecting the measurement of total starch.

Table 2. Total starch values of native starch samples on a wet basis and dry basis obtained by the Megazyme method.

Total Starch		
Sample	**Wet Base (%)**	**Dry Base (%)**
Arracacha	79.08±0.27	90.35±0.30
Hibia	75.72±1.91	83.93±2.13
Chugua	67.39±1.09	75.69±1.22
Cubio	76.13±1.20	83.41±1.31

3.3. Determination of Amylose and Amylopectin Quantities

In the technique for determining the quantities of amylose and amylopectin obtained using the Megazyme method, we can observe in Table 3 that the amounts of amylose are close to 20% for the selected uncommon native starches, which is a similar quantity to that of more common starches such as potato or corn. In the case of this technique, the starches were purified before conducting the test, so it can be ruled out that they have impurities.

Table 3. Amylose content in arracacha, hibia, chugua, and cubio starches. .

Sample	Amylose (%)
Arracacha	17.01±0.56
Hibia	17.56±0.61
Chugua	20.78±2.31
Cubio	25.51±0.41

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310 *3.4. Short-range molecular order*
311 When applying the FTIR-ATR technique to the native starches, graphs with characteristic behavior
312 for starches were obtained, as can be seen in Figure 1.
313 Similarly, the IR wavelengths for the starches fall within the range of more common native starches,
314 which is between 0.6 and 0.8, as shown in the results in Table 4.
315
316 **Table 4.** Ratio of IR radiation at 1045/1022 and 1022/995.
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Sample	IR Ratio 1045/1022(cm^{-1})	IR Ratio 1022/995(cm^{-1})
Arracacha	0,74205	0,82928
Chugua	0,74205	0,84387
Cubio	0,74185	0,85214
Hibia	0,7661	0,84245

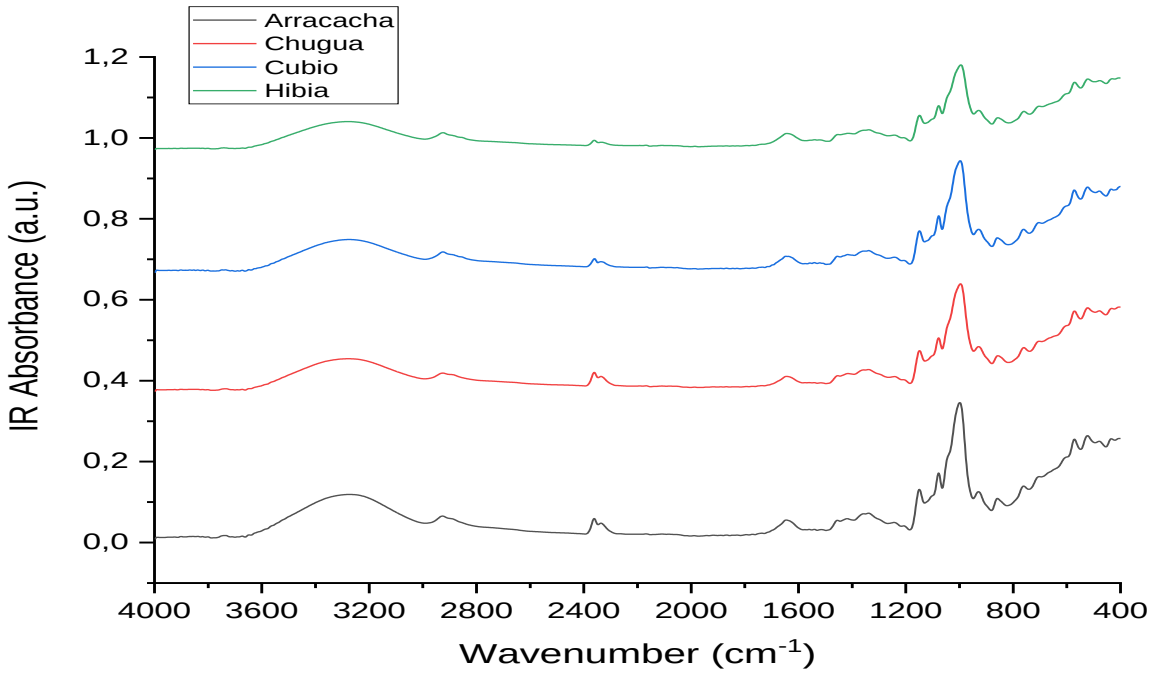


Figure 1. FTIR-ATR spectrum of arracacha (black line), chugua (red line), cubio (blue line), and huia (green line) starches.

3.5. Molecular Weight Determination by Chromatography

In the results of the chromatography study listed in Table 5 for amylose and Table 6 for amylopectin, we can observe that the molecular weight falls between 1 and 2 million g/mol, which are expected quantities for native starches. Therefore, the molecular characteristics of the uncommon starches align with the expectations for this family. The same occurs with the molecular number. In the particular case of arracacha, it presents a slightly lower molecular weight than the other samples; however, it still falls within the parameters of a native starch. Since the molecular weight and molecular number values are so close to each other for all samples, the polydispersity is quite low, indicating minimal variation.

Regarding the molecule radius, it can be observed in the tables that arracacha, despite having a slightly lower molecular weight, has larger molecules compared to the other samples. Conversely, Hibia is the sample with the smallest molecule size.

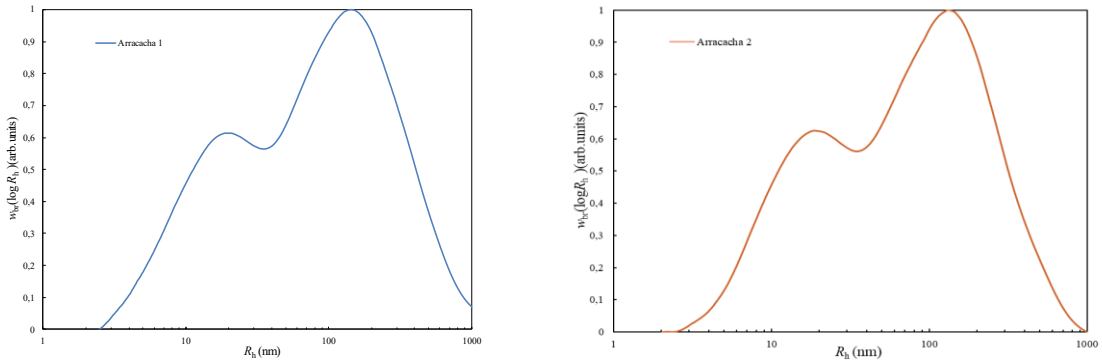
Table 5. Values of molecular number, molecular weight, polydispersity, radius moment, and gyration radius (R_h) obtained by chromatography technique using the MALLS method and 30-3000 columns for the amylose of native starch samples.

Amylose					
Sample	**Molecular number(g/ mol) x10^6**	**Molecular weight(g/ mol) x10^6**	**Polydispersity (Mn/Mw)**	**Radius moments (nm)**	**Average Rh (nm)**
Arracacha	0.73±0.03	0.75±0.01	1.03±0.02	49.45±0.21	19.65±0.03
Hibia	1.414±0.00	1.446±0.01	1.02±0.01	26.15±0.92	18.74±0.02
Chugua	2.423±0.22	2.51±0.26	2.54±2.13	34.45±0.21	22.02±1.14
Cubio	1.576±0.04	1.59±0.06	1.01±0.01	30.05±0.49	25.05±0.46

Table 6. Values of molecular number, molecular weight, polydispersity, radius moment, and gyration radius (R_h) obtained by chromatography technique using the MALLS method and 30-3000 columns for the amylopectin of native starch samples.

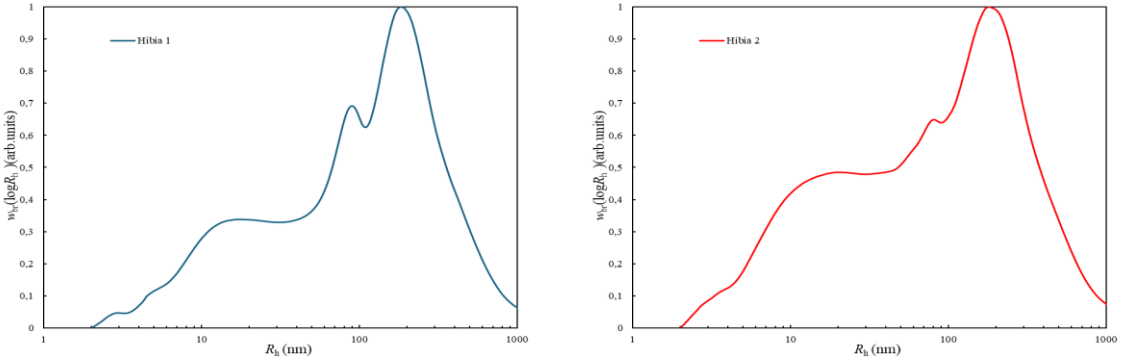
Amylopectin					
Sample	**Molecular number(g/ mol) x10^6**	**Molecular weight(g/ mol) x10^6**	**Polydispersity (Mn/Mw)**	**Radius moments (nm)**	**Average R_h (nm)**
Arracacha	0.76±0.04	2.15±0.15	2.82±0.38	159±3.42	126.64±2.74
Hibia	2.12±0.20	2.85±0.18	1.34±0.04	71.40±1.84	148±2.54
Chugua	1.27±0.13	1.86±0.05	1.48±0.11	77.20±2.33	189.6±2.89
Cubio	1.08±0.03	1.76±0.16	1.62±0.10	65.05±0.49	246.10±4.32

"In the Figures generated by the chromatography technique, we can observe that they tend to have two peaks, one more pronounced than the other. These peaks represent the amylose and amylopectin of the native starches, and their size increases according to the amount of polysaccharide in the sample. This behavior is evident in the graphs of the samples in Figures 2 to 5, both in the original sample and its duplicate. Similarly, when comparing the samples, slight differences in the shape of the peaks can be observed. Specifically, in the shape of the first peak, where in Figure 2, it is much more pronounced and closer to the second peak in the case of Arracacha, whereas in Figure 5, the first peak is much flatter and closer to the axis in the case of Cubio.



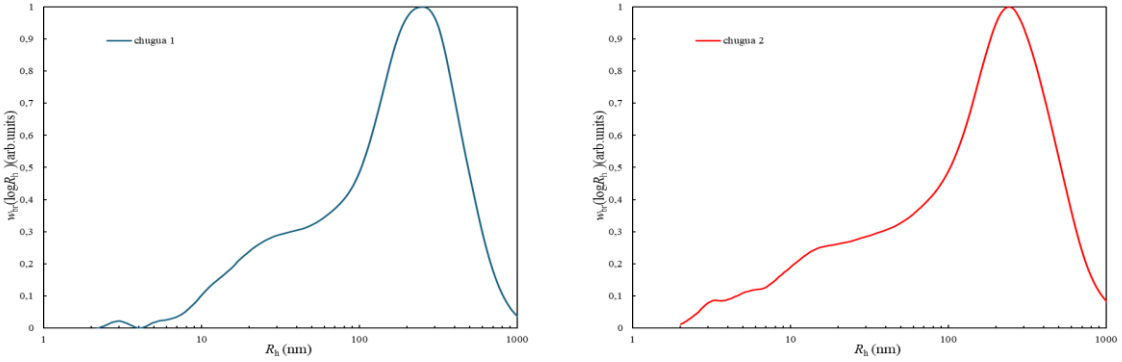
(a) (b)

Figure 2. Graphs of the Arracacha sample using chromatography technique, (a) sample (b) duplicate.



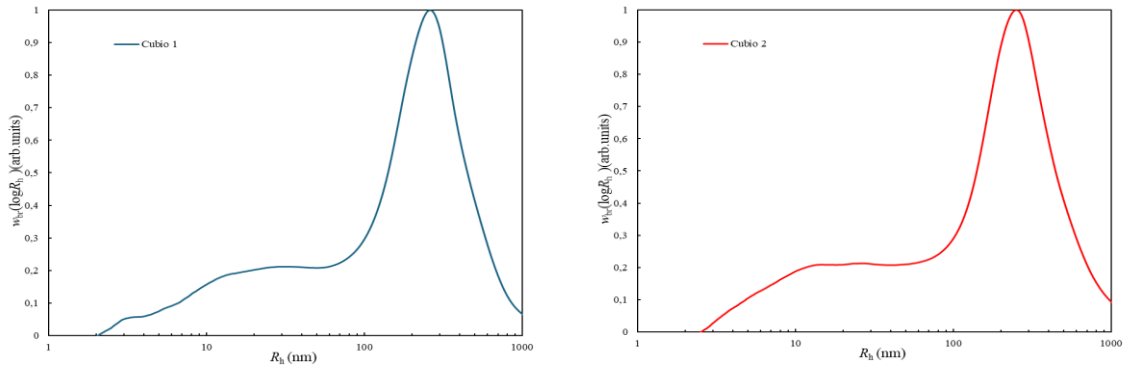
(a) (b)

Figure 3. Graphs of the Hibia sample using chromatography technique, (a) sample (b) duplicate.



(a) (b)

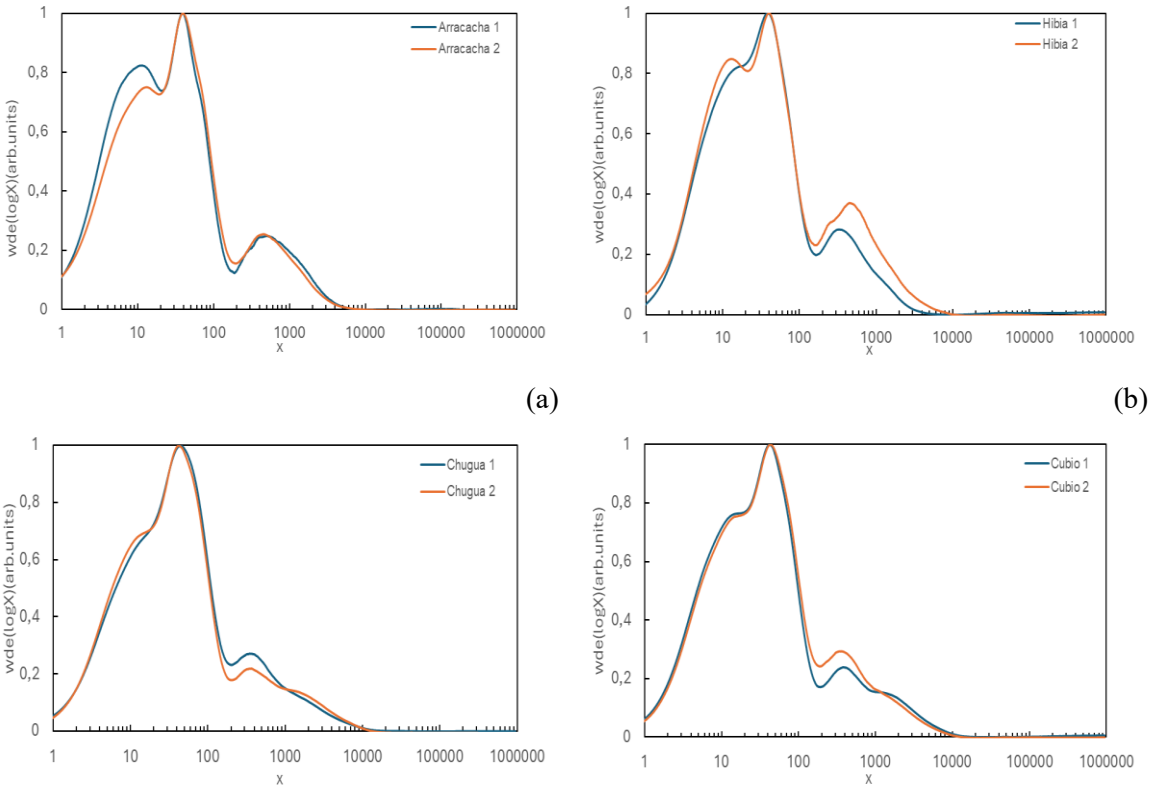
Figure 4. Graphs of the Chugua sample using chromatography technique, (a) sample (b) duplicate.



(a) (b)

Figure 5. Graphs of the Cubio sample using chromatography technique, (a) sample (b) duplicate.

In the case of the data for the debranched starches treated in Origin, we can observe that the behavior of the graphs changed slightly, as seen in Figure 6. It is noticeable how the amylopectin peak is now divided into two peaks, representing polysaccharide chains divided by their size, with one peak for short chains and the other for long chains. Conversely, the peak representing amylose did not undergo significant changes.



(a) (b)

(c) (d)

Figure 6. Graphs of unbranched native starches obtained by the chromatography technique with their respective duplicates, (a) Arracacha, (b) Hibia, (c) Chugua, (d) Cubio.

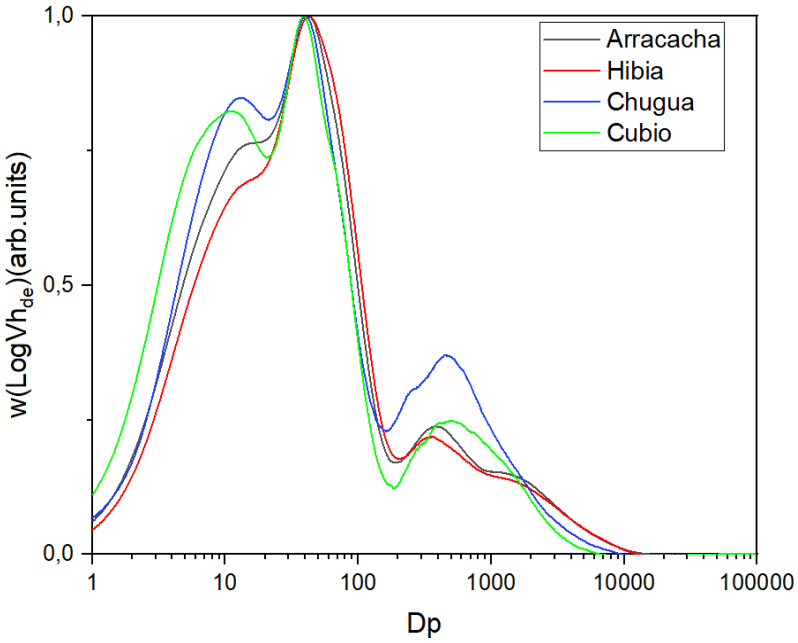


Figure 7. Graphs made in Origin of the unbranched native starches, from which those exhibiting behavior closest to starches were selected.

3.6. Chain Length distribution

Upon examining the results of the processed debranched starches, we observe that the mass fraction tends to be higher in amylopectin for all cases, as shown in Table 7. In all instances, the sum of the percentage of these fractions approaches 100%, indicating that no factors were altering these percentages during the technique application or data processing.

Regarding the degree of polymerization of the samples, it can be observed in Table 7 that amylose exhibits a higher number in the chains compared to amylopectin for all cases. This observation is also evident in the graphs presented in Figures 8 to 11, which graphically represent the chain length of the polysaccharides in the starch samples.

Table 7. Values of mass fraction and degrees of polymerization of unbranched starch samples for amylose and amylopectin obtained by the chain length method.

Amylose	***Amylopectin***

Sample	Mass fraction (%)	Polymerization degree	Mass fraction short (%)	Mass fraction long (%)	Polymerization degree short	Polymerization degree long
Arracacha	14.65±0.21	462±2.62	36.45±1.63	48.85±1.48	12.31±1.41	39.01±0.38
Hibia	20.05±0.64	455.21±2.42	35.50±0.57	44.45±0.07	14.23±1.83	39.63±1.05
Chugua	14.75±0.49	352.97±1.47	49.90±0.85	35.35±1.34	13.36±0.32	43.43±1.81
Cubio	16.90±1.13	390.96±2.63	42.55±0.49	42.55±1.63	14.41±0.45	42.61±0.65

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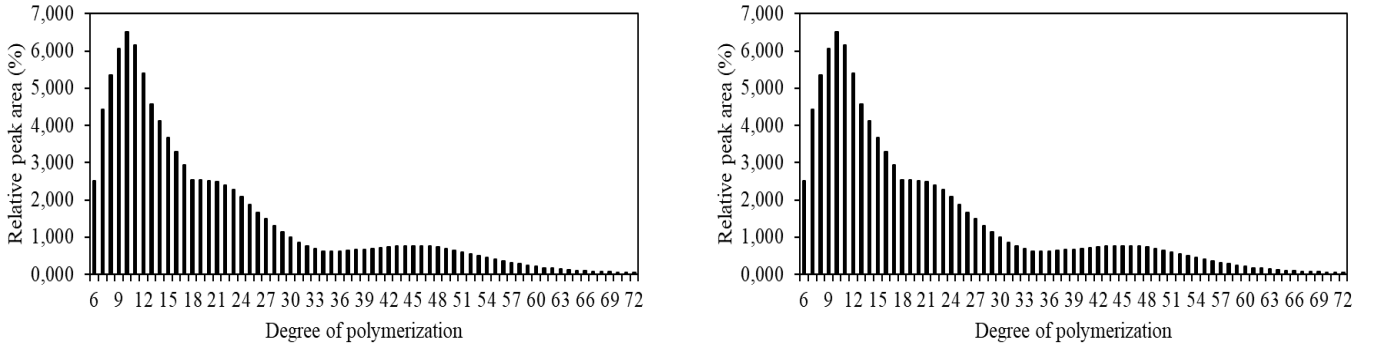

(a) (b)

395 **Figure 8.** Graphs of the degree of polymerization of the Arracacha sample (a) obtained using the
396 chain length technique with its respective duplicate (b).

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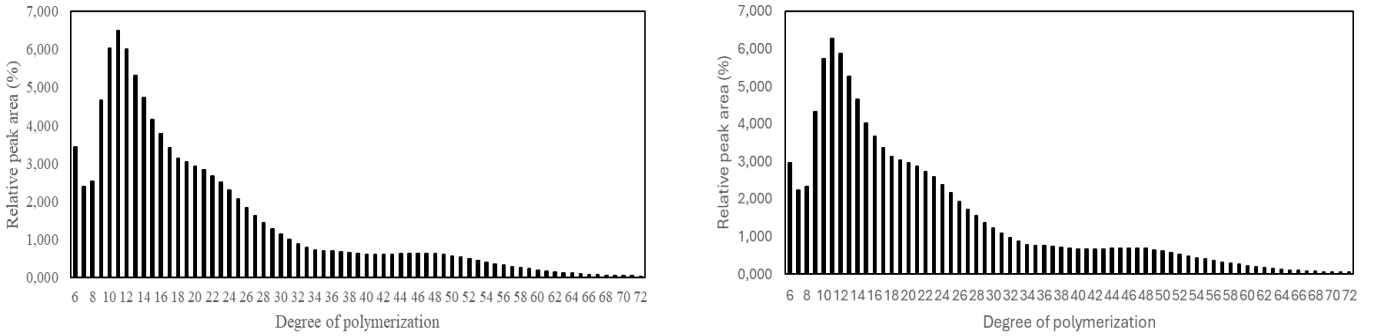
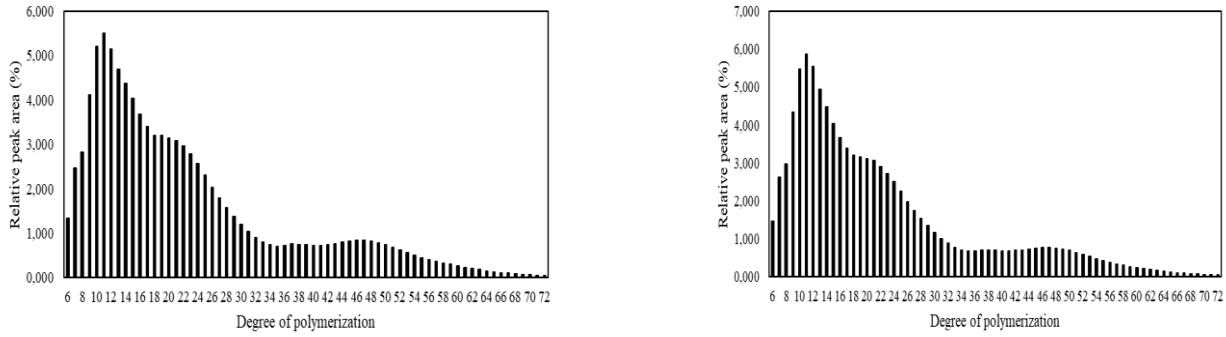


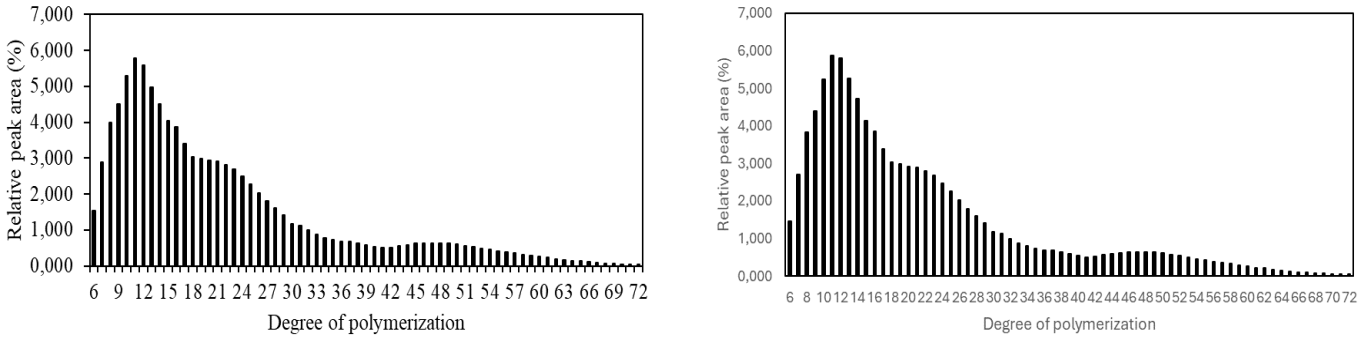
(a) (b)

Figure 9. Graphs of the degree of polymerization of the Hibia sample (a) obtained using the chain length technique with its respective duplicate (b).



(a) (b)

Figure 10. Graphs of the degree of polymerization of the Chugua sample (a) obtained using the chain length technique with its respective duplicate (b).



(a) (b)

Figure 11. Graphs of the degree of polymerization of the Cubio sample (a) obtained using the chain length technique with its respective duplicate (b).

3.7. Thermal properties

In the results of the thermal properties, as observed in Table 8, the peak temperature (Tp) of the selected uncommon starches is around 64 °C for all cases, which is relatively lower than that of other native starches, which is close to 70 °C. While this difference is significant, it is necessary to consider these factors if they are to be used in other tests. In Figure 12, a slightly more detailed behavior of these starches at these temperatures can be observed. Both the sample and its respective duplicate exhibit similar behavior at these temperatures. Additionally, it is observed that no further alterations occur in the calorimetry graph after the melting point is reached.

Table 8. Thermal properties of arracacha, hibia, chugua, and cubio starches.

Sample	To (°C)	Tp (°C)	Te (°C)	GR	ΔH (J ·g^{-1})
Arracacha	61.25±0.3^{a}	64.3±0.3^{a}	71.3±0.1^{a}	10.05±0.1^{a}	11.5±4.3^{a}
Hibia	60.14±0.1^{b}	63.2±0.1^{b}	70.5±0.01^{b}	10.36±0.1^{b}	9.2±3.02^{a}
Chugua	60.67±0.05^{c}	64.3±0.4^{a}	71.3±0.1^{a}	10.69±0.1^{c}	13.6±3.4^{a}
Cubio	62.05±0.1^{d}	64.70±0.2^{c}	71.4±0.1^{a}	9.7±0.1^{d}	6.5±2.31^{b}

T_o = onset temperature; T_p = peak temperature; T_e = end temperature; ΔH = gelatinization enthalpy; GR = gelatinization range (T_e - T_o). Values represent the mean of three determinations ± SE. Means in column not sharing the same uppercase letter are significantly different ($p < 0.05$).

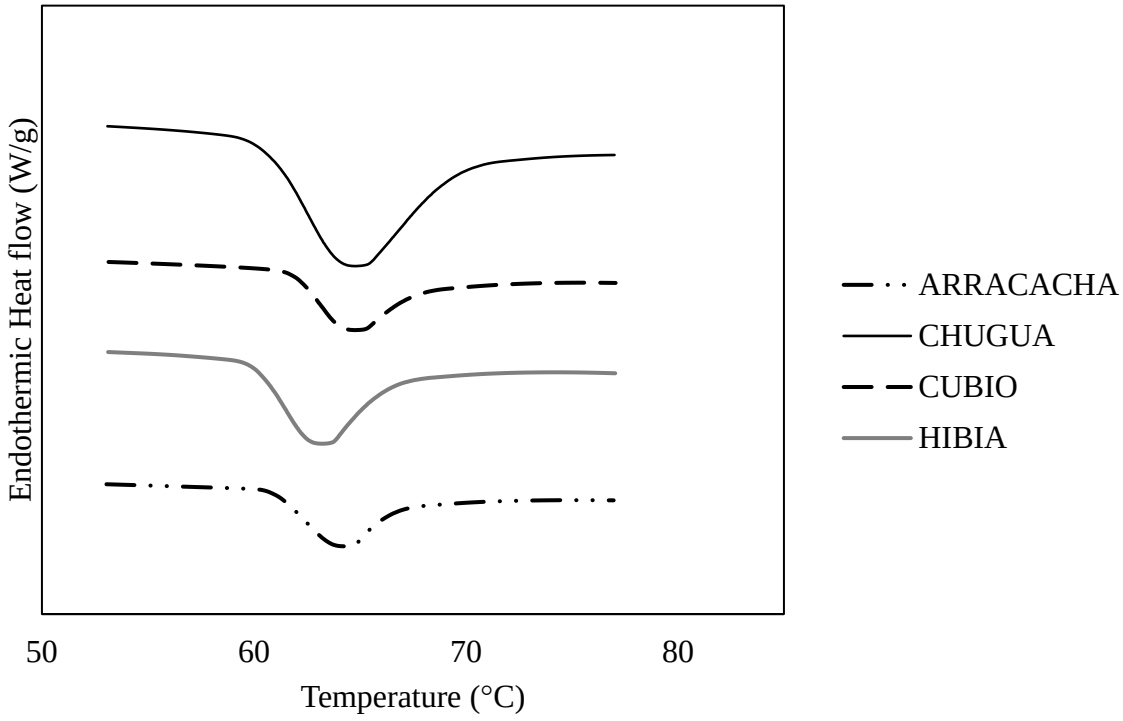
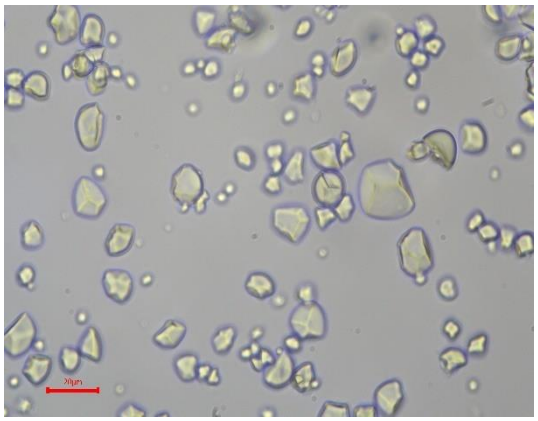


Figure 12. Thermogram of the native starches obtained by the DSC technique.

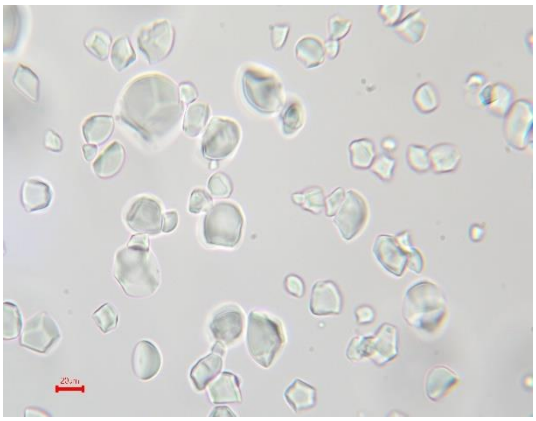
3.8. Optical and Polarized Light Microscopy and SEM

In Figure 13, we can observe the structure of Arracacha granules. A formation close to geometric with defined edges and vertices can be seen, which is uncommon for starches, as they tend to have a more circular shape. However, in the photos under polarized light, extinction crosses can be

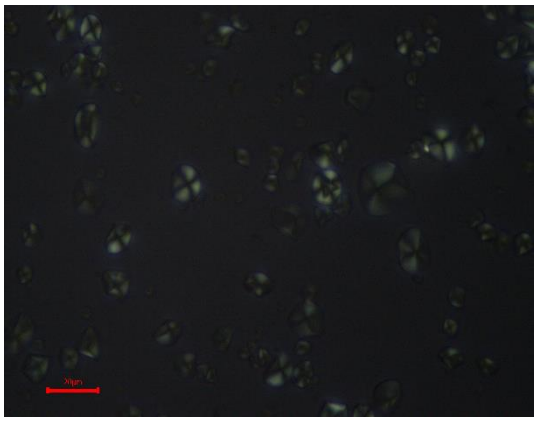
appreciated, which is a characteristic of starches when observed under this light. The geometric structures can also be seen in LV-SEM Figures.



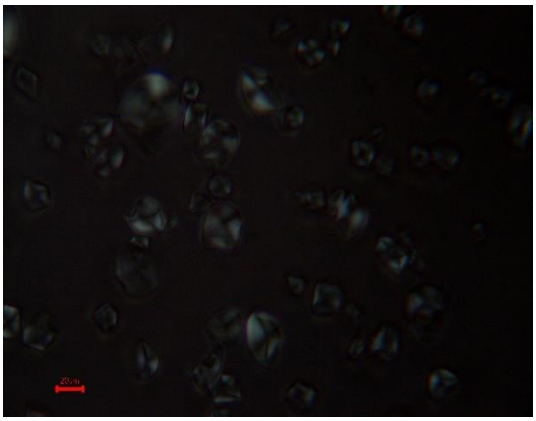
(a)



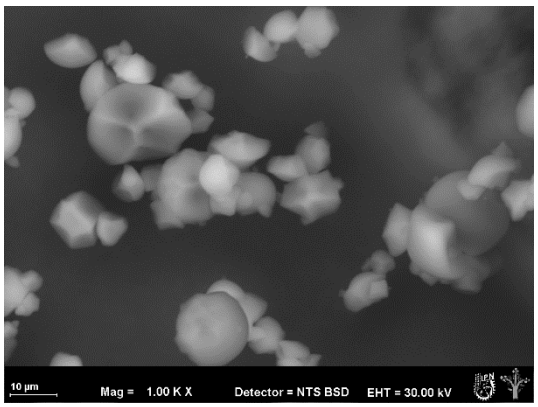
(b)



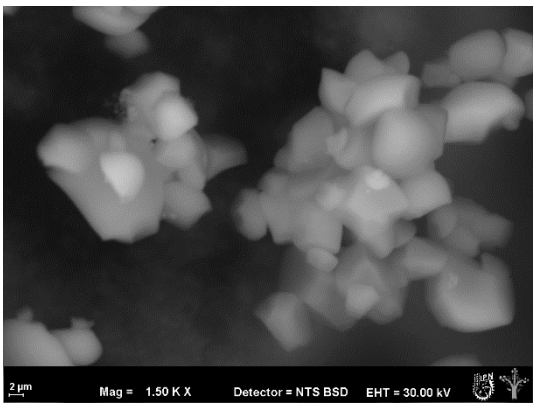
(c)



(d)



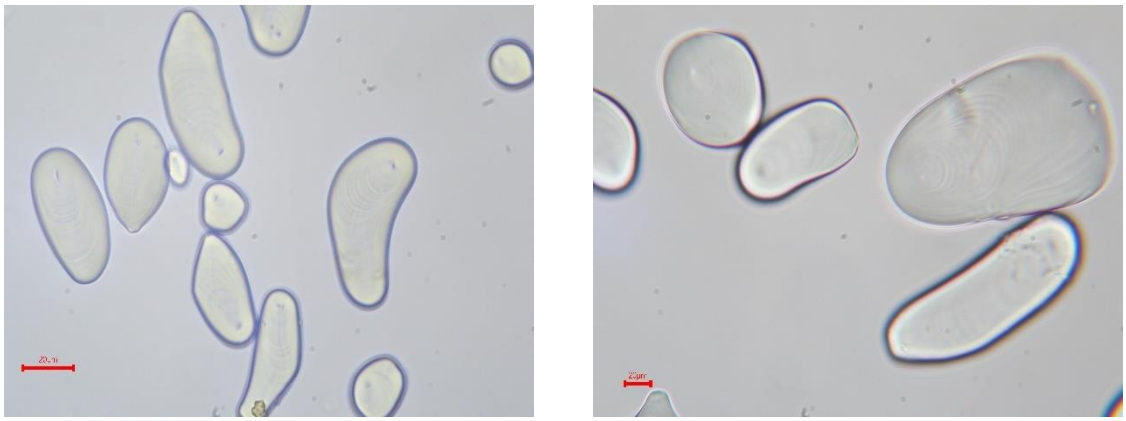
(e)



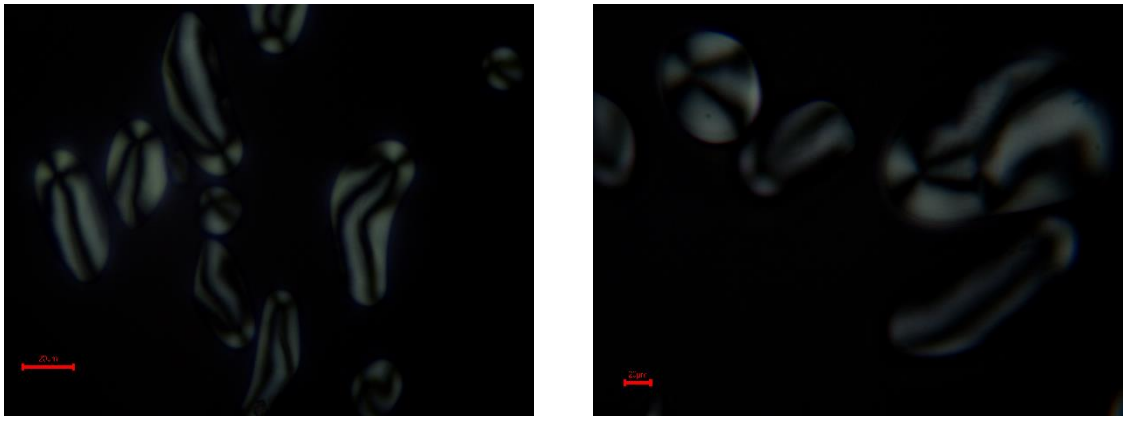
(f)

Figure 13. Micrographics of the Arracacha sample. (a) Optical microscopy at 80x magnification, (b) Optical microscopy at 100x magnification, (c) Polarized light microscopy at 80x magnification, (d) Polarized light microscopy at 100x magnification, (e) and (f) LV-SEM.

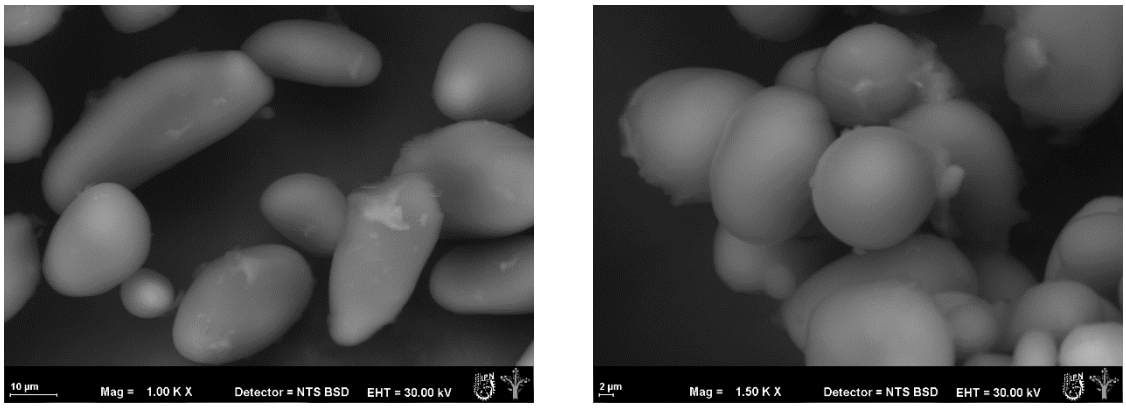
In Figure 14, it can be observed photos of Hibia starch granules and how they have an elongated circular shape resembling an oval, which is close to the shape of more common starches. This is confirmed in SEM technique where these shapes can also be seen. The most notable to mention is the shape of the extinction crosses in polarized light microscopy, which are distorted and twisted due to the shape of the grains. Nevertheless, the characteristic cross for starches can still be identified.



(a) (b)



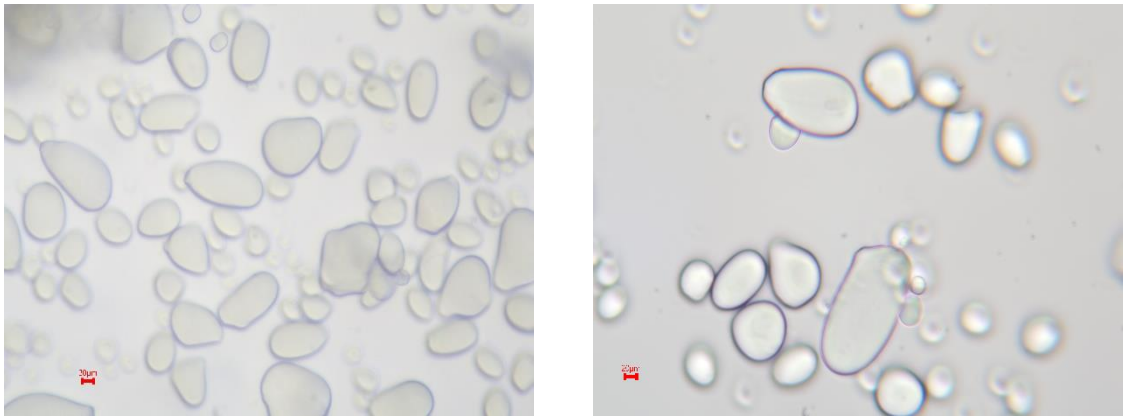
(c) (d)



(e) (f)

Figure 14. Microscopy Figures of the Hibia sample. (a) Optical microscopy at 80x magnification, (b) Optical microscopy at 100x magnification, (c) Polarized light microscopy at 80x magnification, (d) Polarized light microscopy at 100x magnification, (e) and (f) LV-SEM.

In Figure 15, It can be observed the structure of Chugua granules, which have a very similar appearance to that of more common starches. This can be appreciated both in optical microscopy and in SEM. The most notable point to mention is that in polarized light microscopy, it is possible to observe how some of the extinction crosses appear horizontally instead of vertically, which is uncommon.



(a) (b)

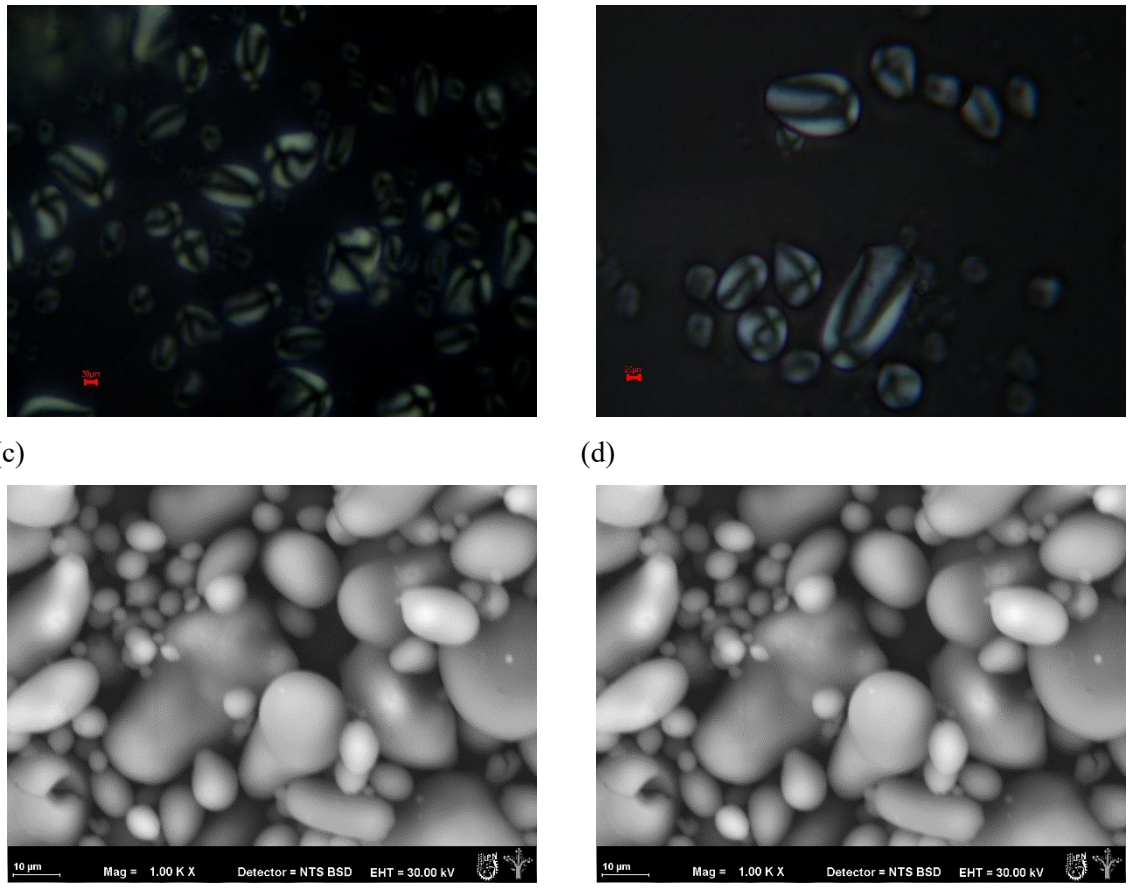
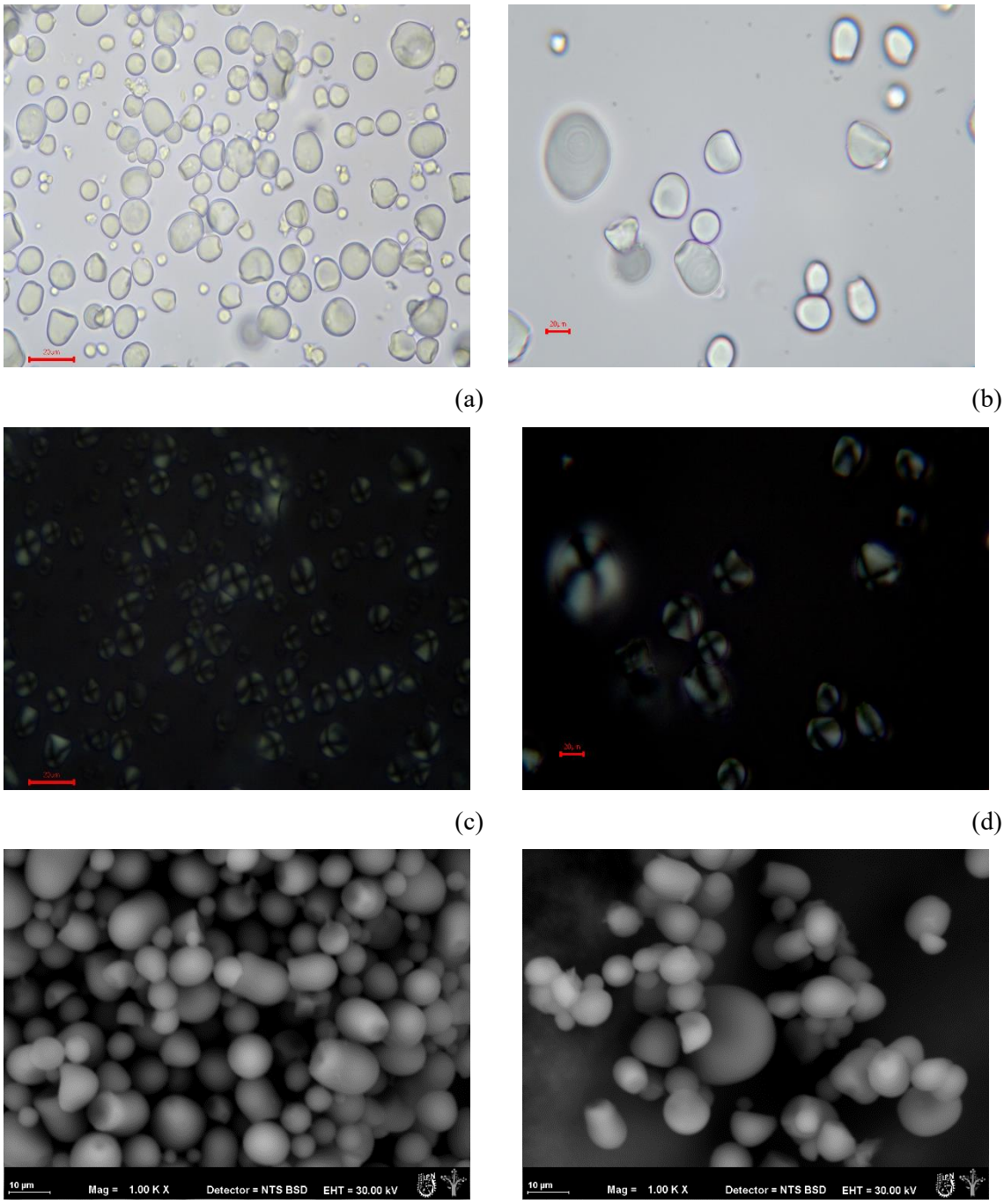


Figure 15. Microscopy Figures of the Chugua sample. (a) Optical microscopy at 80x magnification, (b) Optical microscopy at 100x magnification, (c) Polarized light microscopy at 80x magnification, (d) Polarized light microscopy at 100x magnification, (e) and (f) SEM.

In Figure 16, It can be observed how Cubio exhibits a characteristic appearance similar to common starches, with the difference being that Cubio granules are slightly smaller. In the case of Cubio, no notable characteristics or particularities are observed.



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461 **Figure 16.** Microscopy Figures of the Cubio sample. (a) Optical microscopy at 80x magnification,
462 (b) Optical microscopy at 100x magnification, (c) Polarized light microscopy at 80x magnification,
463 (d) Polarized light microscopy at 100x magnification, (e) and (f) SEM.

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3.10. Digestibility

In Table 9, It can be observed the percentages of different types of starch found during the digestibility technique for raw starches. From this, it can be highlighted that it has a high content of resistant starch, which would provide a significant glucose supply. However, evidence indicates that the digestibility rate K is quite low, indicating that this would take a considerable amount of time. The same can be observed when looking at the percentages of SDS and RDS, which are quite low. This is because the starch was not cooked, making it difficult to digest and utilize the glucose found in the polysaccharide chains. On the other hand, in Table 10, the results for cooked starches are observed. In these, the digestion rate significantly increases, as well as the percentages of RDS and SDS, which are more notable in the initial stages of digestion for glucose extraction. In contrast, the low-resistant starch is lower compared to raw starches. This may be because in the cooking process, samples lose part of these starches in exchange for easier digestibility.

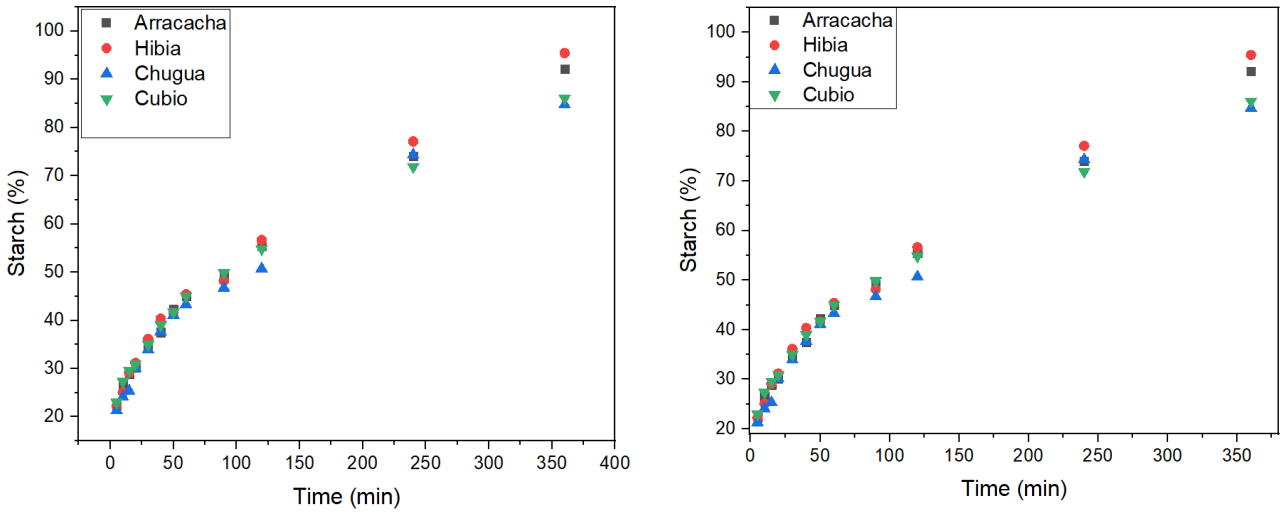
Table 9. Values of resistant starch (RS), rapidly digestible starch (RDS), slowly digestible starch (SDS), and RDS (Krds) and SDS (ksds) rate constants of the raw native starches obtained by the digestibility or kinetics technique.

Raw starch					
Sample	**Krds (%) x10^-1**	**Ksds (%) x10^-1**	**RDS (%)**	**SDS (%)**	**RS (%)**
Arracacha	0.25±0.03	0.02±0.00	16.01 ±0.03	7.57±0.17	76.42±0.21
Hibia	0.09±0.01	0.02±0.03	15.01±0.30	7.52±0.29	77.46±0.01
Chugua	0.01±0.00	0.12±0.03	14.42±0.44	6.56±0.16	79.01±0.27
Cubio	0.02±0.00	0.16±0.02	15.76±0.40	6.49±0.05	77.73±0.46

Table 10. Values of resistant starch (RS), rapidly digestible starch (RDS), slowly digestible starch (SDS), and RDS and SDS rate constants (K) of the cooked native starches obtained by the digestibility or kinetics technique.

Cooked starches					
Sample	**Krds (%) x10^-1**	**Ksds (%) x10^-2**	**RDS (%)**	**SDS (%)**	**RS (%)**
Arracacha	1.31±0.13	0.15±0.00	31.20±0.69	24.67±0.76	44.12±0.07
Hibia	0.61±0.00	0.11±0.00	31.81±0.18	24.30±2.0	43.89±1.89
Chugua	0.59±0.01	0.22±0.00	29.87±0.34	23.65±2.4	46.47±2.78
Cubio	0.08±0.07	0.21±0.00	31.82±1.05	23.55±0.4	44.63±1.47

"When transferring the data to Origin and plotting the graph, a slightly upward curved behavior can be observed over time, as shown in Figure 17 and Figure 18, both for raw and cooked starches. This demonstrates a characteristic behavior for all starches, indicating how the amount of digested starch increases as time progresses.



(a) (b)

Figure 17. Kinetics graphs of digested starch as a function of time (min), (a) raw starch digestion and (b) cooked starch digestion.

4. Conclusions

In conclusion, the investigation comprehensively characterized non-conventional starches from the Andean region of Colombia, including Arracacha (*Arracacia xanthorrhiza*), Hibia (*Oxalis tuberosa*), Chugua (*Ullucus tuberosus*), and Cubio (*Tropaeolum tuberosum*). The findings revealed notable distinctions among the starches, with Arracacha boasting the highest starch purity at 90.3% and varying amylose content ranging from 17% to 25.51%. Moreover, Hibia and Cubio starches exhibited superior short-range order, while Chugua starch demonstrated the highest amylose molecular weight and Hibia starch exhibited the highest amylopectin molecular weight. Morphological analysis showcased unique granular structures for each starch type, with implications for their functional properties. Importantly, our study identified low digestion rates and significant levels of resistant starch in gelatinized forms of these starches, suggesting their potential as valuable alternatives in the food industry. Overall, the physicochemical characterization presented here underscores the nutritional and functional significance of Andean tuber starches, offering promising avenues for further research and application in food science and technology.

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