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Breadmaking Potential of Andean Roots and Tuber Starches from Ahipa (*Pachyrhizus ahipa*), Oca (*Oxalis tuberosa*), and Arracacha (*Arracacia xanthorrhiza*)

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Aim is to explore the breadmaking potential for gluten-free goods of non-conventional starches from Andean crops ahipa, oca, and arracacha. Their characteristics and performance in breadmaking are compared with those of cassava, taken as a reference for conventional gluten-free root starch. Physicochemical properties of breads are studied along with the pasting and thermal properties, composition, and α-amylase hydrolysis of starches. Arracacha starch has the lowest amylose content (2.4%) and the highest water hydration (1.4 g g^{-1}). Its batter shows adequate proofing, but the bread is highly adhesive, with dense crumb. Ahipa starch paste has the lowest peak, trough and final viscosities determined by rapid visco analyzer, and the highest hydrolysis rate (k_{RVA} = 2.30 min^{-1}). Its batter exhibits, along with oca, the highest volume increase during fermentation (193–197%), but structure collapses in the oven and no alveoli are observed in the crumb. Conversely, oca forms a crumb structure similar to cassava, but with higher cell density (131 alveoli cm^{-2}), cohesiveness (0.95), and resilience (0.65) than the latter (71 alveoli cm^{-2}, 0.88, and 0.45, respectively). Oca starch has lower pasting temperature (64 °C) and the starch paste has similar hydrolysis rate (k_{RVA} = 1.92 min^{-1}) compared to cassava (71.9 °C and 2.08 min^{-1}, respectively), making it a suitable option for providing gluten-free yeast-leavened breads with improved technological properties and a comparable glycemic index.

1. Introduction

The search for sustainable food systems has propelled investigations into the extensive biodiversity of ecosystems worldwide. Traditional diets in the countries of the Andean region are a valuable source of commodities that are becoming integrated into the global diet. A clear example has been the pseudocereals like quinoa, amaranth, and so on.[1] Other important sources of nutrients are the roots and tuber crops (R&T), which remain scarcely explored. Among these Andean R&T, ahipa (*Pachyrhizus ahipa*), oca (*Oxalis tuberosa*), and arracacha (*Arracacia xanthorrhiza*) are rich in starch and other health-associated compounds.[2]

Starches have a crucial role in the elaboration of gluten-free (GF) baked goods, and the nature of the starch determines the bread structure.[3] While corn and rice are frequently used in gluten-free (GF) breadmaking, alternative options such as pseudocereals, roots, tubers, and legumes can also be utilized provided there is a sufficient understanding of their functionality.[3] It has been stated that R&T starches, especially cassava starch, provide GF breads with lower volume compared to maize starch but with better texture.[4] The larger size of the root starch granule has been considered responsible of the lower volume, while the better texture was attributed to their lower retrogradation tendency.[4] Given the success of utilizing other unconventional roots and tubers such as taro or malanga in gluten-free breadmaking,[5] it would be valuable to investigate additional unconventional sources like ahipa, oca, and arracacha. This evaluation could potentially boost demand for these ingredients and stimulate their production.

Ahipa plant is leguminous with large roots with starch contents ranging from 44% to 65% db. *P. ahipa* is currently cultivated only in a few pockets of the Andean mountains, although its cultivation declined over the years.[6] Ahipa starch can be extracted with a good level of purity using water in a process mimicking industrial cassava starch extraction at laboratory scale.[7]

Arracacha, also known as white carrot or Peruvian carrot, is a species of the Apiaceae family, cultivated in Central to South America,[2] but with low commercialization and

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Table 1. Proximate composition, physical, chemical and pasting properties, and parameters of the enzymatic hydrolysis of the starch samples.

Sample	Cassava	Ahipa	Oca	Arracacha
Proximate composition				
Moisture content [%]	13.03 ± 0.00 b	8.79 ± 0.14 a	17.83 ± 0.13 c	18.08 ± 0.05 d
Protein [% db]	0.17 ± 0.01 ab	0.26 ± 0.05 b	0.06 ± 0.07 a	0.45 ± 0.04 c
Fat [% db]	0.08 ± 0.05 a	ND	0.11 ± 0.07 a	ND
Ash [% db]	0.11 ± 0.00 a	0.19 ± 0.01 b	0.33 ± 0.00 c	0.52 ± 0.01 d
Total carbohydrates [% db][a)]	99.6 ± 0.1	99.6 ± 0.1	99.5 ± 0.1	99.0 ± 0.0
Total starch [% db]	99.5 ± 0.8 c	94.2 ± 0.8 a	96.6 ± 0.3 b	97.7 ± 0.9 bc
Starch damage [%]	0.43 ± 0.00 a	1.62 ± 0.02 c	0.66 ± 0.00 b	5.41 ± 0.05 d
Amylose [%]	15.5 ± 0.0 c	11.4 ± 1.5 b	11.9 ± 0.3 b	2.4 ± 1.0 a
Physical properties				
Whiteness index	95.6 ± 0.0 c	95.0 ± 0.2 b	93.6 ± 0.2 a	95.6 ± 0.3 c
Mean equivalent diameter [μm]	8.4	6.9	17.3	6.4
WBC [g H_2O g^{-1} starch]	0.84 ± 0.10 a	1.08 ± 0.21 a	0.89 ± 0.09 a	1.40 ± 0.05 b
Thermal properties				
Onset T [°C]	61.7 ± 0.3 c	51.2 ± 0.2 a	56.3 ± 0.5 b	57.3 ± 0.6 b
Peak T [°C]	67.9 ± 0.3 d	57.3 ± 0.6 a	59.7 ± 0.6 b	62.8 ± 0.3 c
End T [°C]	78.1 ± 0.4 d	66.4 ± 0.3 a	68.7 ± 0.3 b	70.2 ± 0.1 c
ΔT [°C]	16.4 ± 0.7	15.2 ± 0.5	12.4 ± 0.8	12.9 ± 0.7
ΔH [J g^{-1}] db	14.1 ± 0.7 b	12.1 ± 0.1 a	13.8 ± 0.3 b	13.7 ± 1.2 ab
Pasting properties				
Pasting T [°C]	71.9 ± 0.0 d	67.0 ± 0.0 b	64.0 ± 0.6 a	69.8 ± 0.5 c
Peak viscosity [cP]	4889 ± 17 b	2764 ± 42 a	6856 ± 30 c	7681 ± 117 d
Trough viscosity [cP]	1677 ± 6 b	982 ± 11 a	2346 ± 32 c	2340 ± 14 c
Breakdown viscosity [cP]	3212 ± 23 b	1782 ± 52 a	4511 ± 62 c	5341 ± 131 d
Final viscosity [cP]	2762 ± 93 b	1538 ± 40 a	3483 ± 30 c	2615 ± 34 b
Setback [cP]	1085 ± 98 c	556 ± 50 b	1137 ± 2 c	275 ± 20 a
Peak time [min]	4.0 ± 0.0 c	4.1 ± 0.0 c	2.8 ± 0.2 a	3.2 ± 0.0 b
Enzymatic hydrolysis parameters				
k_{RVA} [min^{-1}]	2.08 ± 0.02 ab	2.30 ± 0.16 b	1.92 ± 0.08 a	1.83 ± 0.09 a
$\mu_{initial}$ [mPa s]	2461 ± 259 b	1349 ± 288 a	2629 ± 450 b	2325 ± 107 b
μ_{final} [mPa s]	55 ± 5 ab	45 ± 18 a	92 ± 21 b	67 ± 8 ab
μ_{∞}	33 ± 6 a	37 ± 5 a	84 ± 3 c	54 ± 8 b

Results are expressed as mean ± SD. Different letters within a row indicate significant differences ($p < 0.05$). db, dry basis; ND, not detectable. [a)] Calculated by difference with the protein, fat, and ash contents.

industrialization.[8] The root has 55–64% of starch (db)[8] and can be processed peeled or unpeeled for starch extraction. Starches from peeled roots have similar or slightly lower ash, lipid, and protein contents[9,10] than those from unpeeled roots.[8,11]

Oca is a plant from the Oxalidaceae family, native to the Andes, with starch contents around 60% db.[12] Oca tuber is becoming popular in New Zealand and Mexico but is not exploited at industrial level.[13] The small size and the irregular surface of the tubers make them hard to peel, thus they are usually processed unpeeled.[9,14,15]

Considering the limited literature available about the starch from those unconventional materials, this work aimed to study the performance of ahipa, oca, and arracacha starches as ingredients for gluten-free yeast-leavened bread compared to cassava as a reference for GF root starch, and relate it to their physicochemical, thermal and pasting properties, and their susceptibility to hydrolysis with α-amylase.

2. Results and Discussion

2.1. Starch Composition and Color

The composition and properties of the starches extracted from oca, arracacha, and ahipa, and a commercial cassava starch are shown in **Table 1**.

Cassava starch is characterized for its high whiteness, which is partly associated to the low content of fat, proteins, and colored compounds in this root. Arracacha starch showed similar whiteness index to that of cassava. Oca and ahipa resulted slightly lower, but still in values associated with good quality starches (Table 1). The whiteness of the samples agrees with their high purity (total starch contents >94%). This is expected for root and tuber starches, as they typically have low protein, ash, and lipid content.[16] Regarding the amylose content, cassava and ahipa were within the typical values reported for these

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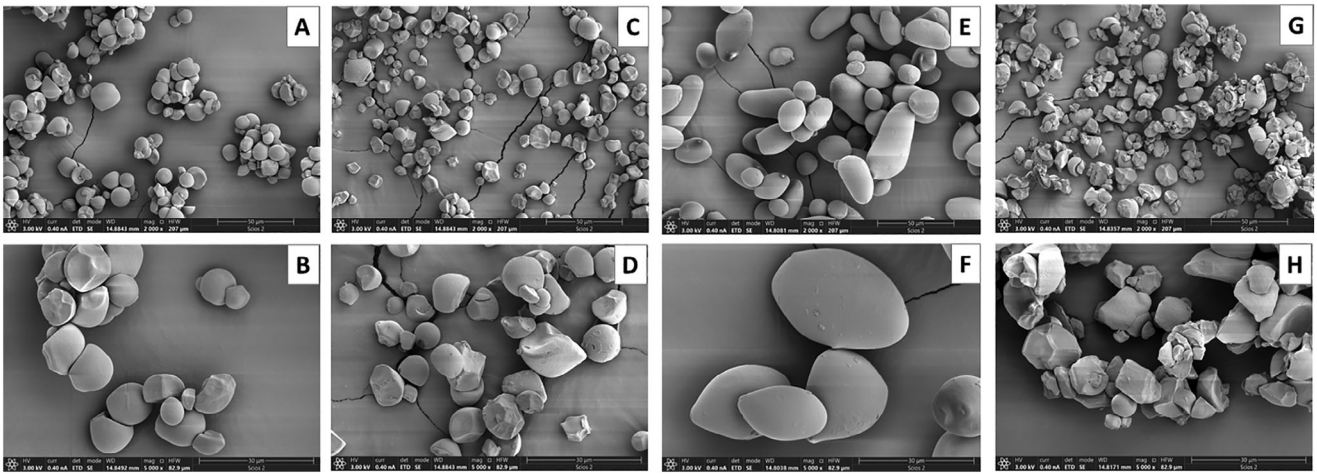

Figure 1. SEM images of starches at magnifications of 2000× (upper row) and 5000× (lower row): A) and B) cassava; C) and D) ahipa; E) and F) oca; and G) and H) arracacha.

starches.[17,18] Oca starch showed total amylose content like ahipa (Table 1) but lower than the apparent amylose value (determined by iodine affinity) reported by Santacruz et al.[19] for oca starch from Ecuador (17.4–19.4%). Higher amylose values have been reported for oca starches from Argentina, Peru, and New Zealand (21.4–28.2%).[12–14,19] Arracacha has significantly low amylose content, even lower than one previously reported (3–5%).[19]

Regarding the granule size and morphology, ahipa starch was smaller than cassava (Table 1). Both starches showed spherical granules with truncated ends, but ahipa also exhibited irregular shaped granules, which might indicate more compact structure within the root (**Figure 1**). Oca starch was composed of smooth surfaced granules, showing a variety of shapes that went from spherical, elliptical, and elongated ones, leading to higher granule size. Even values in the literature confirmed that variation.[9,20] Arracacha starch had the smallest, with very irregular granules that seem to be fractured structures (Figure 1). Conversely, Castanha et al.,[8] and Londoño-Restrepo et al.,[11] reported higher granule sizes, ranging between 8 and 20 µm, but the latter also showed micrographs in which the granules appear as ovoid particles formed by 6–10 wedge-shaped assembled granules of sizes between 4 and 12 µm. In the present work, ovoid structures were scarcely observed, with predominance of the wedge-shaped granules. Divergence could be explained considering that these granules are fragile and could be fragmented during the isolation. In fact, the high starch damage percentage and WBC of arracacha starch (Table 1) support the fragility of that starch. For all starch samples, the starch damage percentage was positively correlated with the WBC ($r = 0.981$, $p = 0.0189$), and negatively correlated with the amylose content of the starch ($r = -0.962$, $p = 0.0383$), which could be useful in the selection of the starches.

2.2. Thermal Parameters of Starches

Thermal parameters of the starches are shown in Table 1. Ahipa showed the lowest gelatinization temperature (57.3 °C), lower than other reported values (60.0–69.1 °C),[7,21,22] and besides the arracacha it showed the lowest enthalpy. Enthalpy values reported for arracacha starch are rather variable (2.1–17.6 J g^{-1}),[8,10,11,19] indicating either differences in the characteristics of starches isolated from different root cultivars or granule damage produced during the extraction methods. Oca showed the narrowest gelatinization temperature range, as indicated the ΔT in Table 1, compared to the other starches, with a peak temperature and gelatinization enthalpy in the range of those reported in previous studies.[13,19]

2.3. Pasting Performance and Enzymatic Hydrolysis of Starches

The behavior of the hydrated starch during cooking was assessed through the pasting performance (**Figure 2**). Cassava and ahipa starches led to lower viscosity through heating and cooling, particularly the gelatinization of ahipa starch results in low viscosity. In consequence, ahipa starch showed peak, trough, and final viscosities considerably lower than the other starches (Table 1). The oca starch showed the lowest pasting temperature, despite having intermediate gelatinization temperatures compared with the other starches. Since the pasting temperature is the minimum one required to increase the viscosity, oca starch is giving earlier increase of the viscosity, which could be related with the higher granule size compared to the other starches. An early gelatinization was also reported for potato starch that led to high gel force measured, attributed to the increased granule size and fast water uptake of this starch.[23] Oca starch gave broader viscosity peak after heating. It seems quite plausible that the diverse granule size distribution observed in Figure 1 for this starch explain that the gelatinization was spanning for longer. The starch from arracacha gave the highest peak viscosity (Table 1), which has been previously reported. The high viscosity of arracacha starch could be attributed to its high potassium content[11] or phosphorous content.[24] Regarding phosphorous, similar results have been observed with potato starch, in which the increased phosphorus content enhances the granules swelling and thus higher peak viscosity of the starch paste,[23] but that could not explain the viscosity of oca starch that

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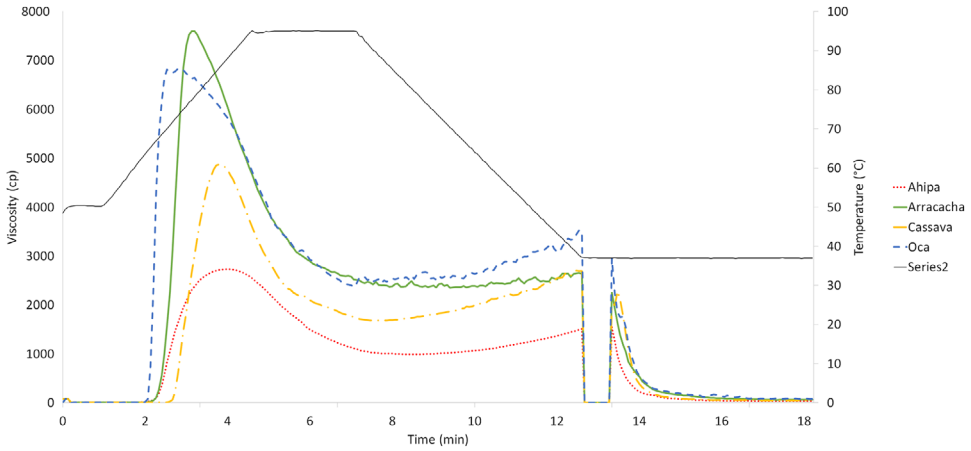

Figure 2. RVA curves of the starch pastes comprising the digestogram with α-amylase to evaluate the starch enzymatic hydrolysis. The break of the curve between minutes 12.8 and 13.3 corresponds to the interruption of stirring for the addition of the α-amylase enzyme. After that addition, the viscosity decrease corresponds to the enzymatic hydrolysis curves (digestogram).

also contains phosphorus,[25] thus while the phosphorus content is directly related to the swelling capacity of a starch, it is not the sole determinant. Arracacha and ahipa starches, despite showing similar size, but the phosphorus content of arracacha, besides its high protein and ash content and low amylose content might explain their different pasting performance.

The fragility of the arracacha starch, previously mentioned, was also observed during cooking, giving a high breakdown value (Table 1). Conversely, it showed the lowest setback, which was expected considering the low amylose content and therefore limited availability of amylose chains to be reorganized. Furthermore, the setback of the analyzed starches was negatively correlated with the WBC (−0.957, $p = 0.0433$), showing oca and cassava starches the highest setback values (Table 1).

The pasting properties evaluation was followed by the enzymatic hydrolysis assay (digestograms) previously reported by Santamaria et al.[26] (Figure 2). The addition of the amylase promoted a rapid decrease of the paste viscosity, but significant differences were observed depending on the type of starch (Figure 2, Table 1). Highest hydrolysis kinetics constant (k_{RVA}) was observed for ahipa starch gel, with no significant difference with cassava starch (Table 1). This susceptibility to be enzymatically hydrolyzed could be related to its low viscosity, which favors the enzyme diffusion and access to the starch chains.[27] Noda et al.,[28] reported an inverse correlation between hydrolysis rate of native root and tuber starches (potato, sweet potato, cassava, and yam) and their pasting properties such as peak viscosity and breakdown, but no correlation was observed for the gelatinized starches. Conversely, in the present work a significant negative correlation was found between the k_{RVA} values and the peak viscosity and the breakdown of starch pastes ($r = -0.999$, $p = 0.0014$, and $r = -0.997$, $p = 0.0028$, respectively).

2.4. Breadmaking Performance of Starches

2.4.1. Batter Volume Increase during Fermentation

The kinetics of volume increase during fermentation of the starch batters (triggered by sucrose addition) were recorded (**Figure 3**), because the batter ability to retain gas during both, fermentation and cooking, are crucial for determining the loaf volume. Surprisingly, cassava starch gave the slower and lowest yeast fermentation, reaching a much lower plateau than the other starches. Ahipa and oca starches showed faster fermentation, reaching earlier a much higher plateau. Arracacha starch led to an intermediate proofing kinetics.

2.4.2. Bread Volume and Crumb Structure

The results after baking showed different pattern from that of the batter fermentation. Breads obtained from cassava and oca starches had the highest volume (**Table 2**). Therefore, despite the slow fermentation, cassava was able to produce high-volume breads. Likely, the viscosity of the system after starch gelatinization was sufficient to expand and retain the gas released during initial steps of baking. The bread volume was positively correlated with the setback viscosity in the RVA assays ($r = 0.997$, $p = 0.0027$). The increase in viscosity as the temperature decreases might be linked to the quick stabilization of the alveolar structure once the bread is removed from the oven, helping

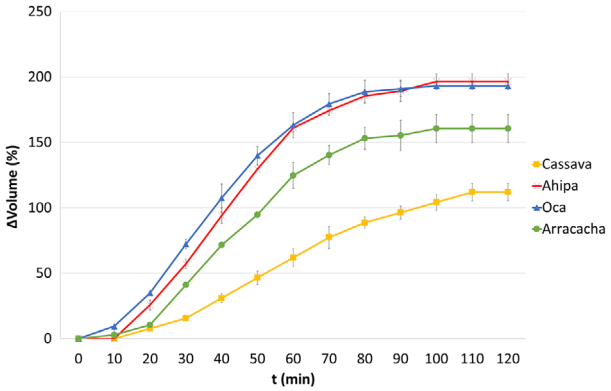

Figure 3. Volume increase during fermentation at 30 °C of the bread batters obtained from different starches.

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Table 2. Physical characteristics of bread and crumb and TPA parameters of the crumb.

		Cassava	Ahipa	Oca	Arracacha
Bread	Moisture [%]	47.9 ± 0.1 a	52.2 ± 0.4 b	52.7 ± 0.1 b	62.7 ± 0.3 c
	Volume (cm^3)	19.7 ± 0.9 c	12.2 ± 0.8 b	20.5 ± 0.6 c	10.6 ± 0.8 a
Crumb	Color				
	*L**	81.77 ± 2.38 c	63.58 ± 1.61 a	71.99 ± 1.96 b	65.60 ± 2.58 a
	*a**	-0.70 ± 0.06 a	3.35 ± 0.51 d	0.24 ± 0.10 c	-0.22 ± 0.05 b
	*b**	6.06 ± 0.94 a	13.08 ± 0.94 b	13.38 ± 0.59 b	14.11 ± 0.69 b
	Internal structure				
	Cell density [alveoli cm^{-2}]	71 ± 11 a	N/D	131 ± 19 b	N/D
	Cell size [mm^{-2}]	0.45 ± 0.08 a	N/D	0.30 ± 0.10 a	N/D
	TPA parameters				
	Hardness [N]	2.1 ± 0.3 a	2.7 ± 0.4 bc	2.3 ± 0.2 ab	1.9 ± 0.2 a
	Adhesiveness [N s]	0.1 ± 0.2 a	0.6 ± 0.4 a	0.0 ± 0.0 a	2.8 ± 0.9 b
	Springiness	1.00 ± 0.01 b	0.77 ± 0.16 a	0.92 ± 0.13 ab	0.92 ± 0.02 ab
	Cohesiveness	0.88 ± 0.03 b	0.69 ± 0.04 a	0.95 ± 0.00 c	0.86 ± 0.02 b
	Chewiness [N]	1.9 ± 0.2 ab	1.5 ± 0.5 ab	2.0 ± 0.3 b	1.5 ± 0.2 a
	Resilience	0.45 ± 0.01 b	0.31 ± 0.02 a	0.65 ± 0.13 c	0.43 ± 0.02 b

Different letters within a row indicate significant differences ($p < 0.05$). N/D, not determined.

to preserve the crumb structure. Additionally, bread volume was negatively correlated with the WBC of the starch ($r = -0.983$, $p = 0.0171$). Arracacha and ahipa starches showed very good fermentation but the structures collapsed in the oven, resulting in low bread volumes, being arracacha the lowest. As observed in **Figure 4**, the cross section of the ahipa and arracacha breads shows that the structure is compact with no evident alveolar distribution; in opposition to cassava and oca that formed a structured crumb. It must be also highlighted that arracacha bread retained a considerable amount of water after baking, resulting in very high moisture content, which might be related with its higher WBC.

In both, cassava and oca, some extremely enlarged gas cells were observed in the crumb, indicating some coalescence of the gas cells (Figure 4). Those big holes were excluded when performing the crumb analysis. Normally, lower size of the cells, uniformly distributed, is a desirable structure for a well-developed crumb.[29] In this sense, oca gave better aerated breadcrumb than cassava because it showed similar cell size but a significantly higher ($p < 0.05$) cell density (Table 2). This could be partially explained by the higher viscosity reached by oca starch compared to cassava starch, which favors gas retention.

2.4.3. Bread Color and Texture

Casava starch bread had whiter crumb, with the highest *L** and lowest *a** and *b** coordinates (Table 2). Ahipa, oca, and arracacha tend to yellow, indicated by a positive *b** value, but ahipa also has a red hue, indicated by a positive *a** value which provides a final dark orange tone (Figure 4). Regarding the textural parameters of the crumb (Table 2), the low cohesiveness and chewiness of ahipa bread indicates a fragile structure which might be related to the

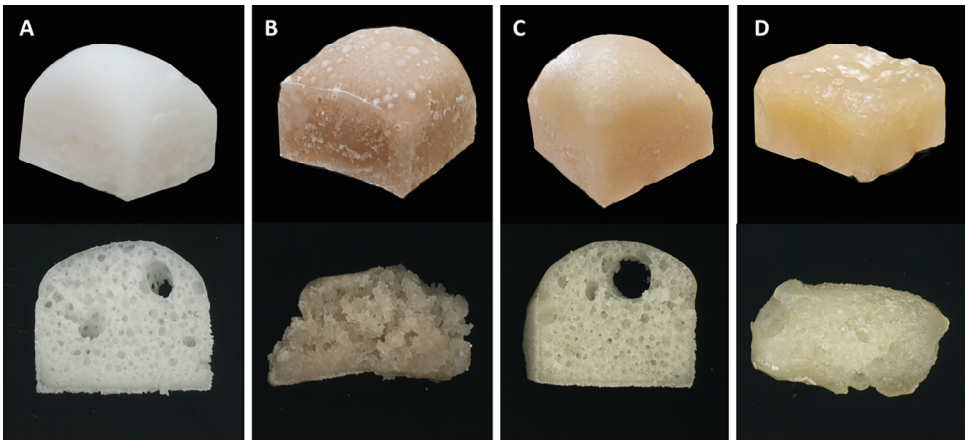

Figure 4. Bread appearance (upper row) and cross section (lower row) of gluten free breads made from: A) cassava; B) ahipa; C) oca; and D) arracacha starches.

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low gas retention and low volume of this bread. This behavior is probably associated to the weak pastes formed by this starch since cohesiveness and resilience of the starch breads resulted positively correlated with the final viscosity in the RVA ($r = 0.991$, $p = 0.0093$ and $r = 0.957$, $p = 0.0427$, respectively). Chewiness was positively correlated ($r = 0.956$, $p = 0.0435$) with the setback of the starch paste. Increased viscosity might be related to a firmer crumb structure, requiring more mastication before swallowing. Additionally, a positive correlation ($r = 0.975$, $p = 0.0249$) between the gelatinization enthalpy of the starch and the springiness of the resultant bread was observed.

Arracacha behaved similarly to cassava in most of the analyzed TPA parameters but showed a considerably increased adhesiveness compared to the other samples, likely attributed to its high moisture content (Table 2). For all the starch samples, adhesiveness was positively correlated with the WBC ($r = 0.971$, $p = 0.0288$) and starch damage ($r = 0.997$, $p = 0.0028$), and negatively correlated with bread volume ($r = -0.966$, $p = 0.0344$). Oca was also similar to cassava in most of the parameters, but exhibited improved cohesiveness and resilience, highest among all the analyzed samples. This is an important feature in gluten-free bakery products since lack of cohesiveness is one of the main disadvantages compared to wheat breads, which is reflected in an undesirable crumbly texture.

3. Conclusions

Ahipa, oca, and arracacha starches were extracted on a laboratory scale in good purity (>94%) without any refining step. All starches were low in amylose, particularly the arracacha starch (2.4%). These unconventional starches differed in their microstructure and physical properties, particularly thermal and pasting behavior. All of them showed lower gelatinization temperature than cassava. Oca and arracacha also showed high viscosity during heating and cooling. Regarding the breadmaking potential, oca showed good proofing behavior, leading to a yeast-leavened bread with similar volume than the one made with cassava starch. Both, oca and cassava produced a structured crumb, but oca also showed improved alveolar density, cohesiveness, and resilience. These texture parameters were positively correlated with the final viscosity in the RVA assay. Arracacha starch showed similar final viscosity to cassava, and its bread crumb showed similar cohesiveness and resilience. However, the high percentage of damaged starch and increased WBC of arracacha led to breads with no structured crumb, and high moisture and adhesiveness. Ahipa starch also failed to form a structured crumb, which is likely due to the low viscosity of its starch paste.

These results suggest that oca starch could be a new ingredient for the development of gluten free starch breads, with digestibility similar to that of cassava starch, and improved technological properties.

4. Experimental Section

Ahipa (*P. ahipa* Wedd Parodi) plants were cultivated at Paraje Esperanza, Misiones, Argentine. Two-year plants were harvested, and the roots were immediately sent to the lab. Oca (*O. tuberosa*) tubers from the yellow variety *Bola kamusa* and arracacha (*A. xanthorrhiza* Bancroft) roots from "White" variety were purchased at a local market in Quito, Ecuador.

The roots and tubers (R&T) were brushed under running tap water to remove any soil rests, disinfected by immersion in a 250 ppm chlorine solution for 10 min at room temperature, and air dried on the bench for 12 h. Ahipa peel was removed by hand, and arracacha roots were peeled using a potato peeler. Oca tubers were processed unpeeled.

Starch Extraction: Starch was extracted following the protocol described in Díaz et al.,[7] based on the industrial cassava starch extraction process. In brief, the R&T were diced into 1 cm pieces, soaked with tap water (1 L kg^{-1}), and ground using a domestic mixer (DHB-675, Daewoo, China). The mixture was left for 24 h at 4 °C and filtered using a cotton cloth. The slurry was left to sediment for 24 h at 4 °C and washed. The procedure was repeated until the filtration supernatant was translucent (five extractions for oca and six extractions for ahipa and arracacha). Sediments were blended and poured onto stainless steel trays, dried in a convection oven at 40 °C, milled, and sieved though 100 mesh (0.149 µm). The powders were stored in sealed containers until used.

A commercial cassava starch (Karay, Zico S.C.C., Pifo, Ecuador) sieved through 100 mesh was used as a reference.

Physical and Chemical Characterization of Starch: The moisture content was determined gravimetrically in a Kern DBS60-3 moisture analyzer (Balingen, Germany). The protein content was determined by the Dumas method in a rapid N exceed Nitrogen analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) using 6.25 as nitrogen-to-protein conversion factor. Total fat was quantified in a Soxtec 8000 equipment (FOSS Analytical Co., Ltd., Suzhou, P.R. China) using hexane as solvent,[30] and ashes were determined at 550 °C.[31] Total carbohydrates percentage was calculated by subtracting the percentages of ash, lipids, proteins, and moisture.

Total starch, amylose content, and starch damage percentage were determined using the assay kits K-TSTA, K-AMYL, and K-SDAM (Megazyme, Bray, Ireland), respectively. Results were expressed as % w/w.

Starch color coordinates L^*, a^*, and b^* from the CIELAB space were determined using a CR400 Konica Minolta colorimeter (Osaka, Japan). The whiteness index was calculated as:

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

The morphology of the starch granules was analyzed in a SCIOS 2 focused ion beam scanning electron microscope at 10 kV. Micrographs were taken at 2000 and 5000× magnification. Granule size distribution was analyzed from the micrographs using the NIS-Elements Imaging software (Nikon Inc., Tokio, Japan). At least 200 granules were analyzed from four or more different images.

Thermal properties of the starches were analyzed by DSC (Q2000, TA Instruments, Inc., New Castle, Delaware, USA). Starch samples (7 mg) were weighed in stainless steel pans and suspended in distilled water at a ratio of 1:4 (starch:water, w:w). The analysis was performed against an empty pan, from 25 to 140 °C at a ratio of 5 °C min^{-1}. Onset temperature, peak temperature, and gelatinization enthalpy (J g^{-1} db) were calculated from the thermograms using the equipment software (Universal Analysis 2000, vs 4.5 A, TA Instruments – Waters LCC). Three replicates were performed for obtaining the average values.

Starch pasting properties and digestibility were evaluated according to Santamaria et al.,[26] using a RVA 4500 model Rapid Visco Analyzer (Perten Instruments, Macquarie Park, NSW, Australia). The RVA settings were: 50 °C for 1 min, heating from 50 to 95 °C at 10 °C min^{-1}, holding at 95 °C for 2.5 min, cooling down to 37 °C at 10 °C min^{-1}, followed by holding at 37 °C for 36 s for the addition of 0.1 mL VI-B α-amylase from porcine pancreas (900 U mL^{-1}, EC 3.2.1.1, Sigma Aldrich, Sigma Chemical, St. Louis, MO, USA). The viscosity continued to be recorded for 5 min at 37 °C. The pasting parameters comprised pasting temperature (°C), at which starch viscosity started to increase, peak viscosity, trough viscosity, breakdown,

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setback, and final viscosity. The hydrolysis curves were modeled by a first order kinetics as described in Santamaria et al.:[26]

$$\mu = \mu_{final} + (\mu_{initial} - \mu_{final})\, e^{-kRVAt} \quad (2)$$

where μ the apparent viscosity (mPa s) at each time point, $\mu_{initial}$ was the viscosity registered immediately after adding the enzyme, and μ_{final} was that at the end of the assay, k_{RVA} (min^{-1}) was the kinetic constant, reflecting the hydrolysis rate, t (min) was the hydrolysis time, and $\mu\infty$ was the final viscosity obtained from the modeled curve. The analysis was performed at least in duplicate.

The water binding capacity (WBC) of each starch, expressed as g water g^{-1} sample, was determined according to the AACC method 56-30.01.[32]

Breadmaking Process: The batter was formulated according to Espinosa-Ramírez et al.[33] For every 100 g of starch, 1.5 g of salt, 1.5 g of sugar, and 1 g of dried yeast were used. The amount of water was determined by the WBC of the respective starch. The water was mixed with the yeast and equilibrated to 30 °C for 10 min. The starch, salt, and sugar were mixed and then yeast suspension was added. The batter was mixed using a EURO-ST 40 digital stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 35 rpm for 10 min. Fermentation curves were determined pouring 2 mL of batter (Vi) in a 15 mL graduated cylinder, which was kept at 30 °C. The batter volume was measured every 10 min for 2 h (Vt). Data were expressed as percentage of volume increase:

$$\Delta V\,(\%) = (Vt - Vi)\,/Vi \times 100 \quad (3)$$

Batter aliquots (10.0 ± 0.2 g) were added to 3 cm cubic silicon molds and fermented for 1 h at 30 °C, baked for 12 min at 130 °C without convection. After cooling down, breads were stored in sealed bags until used. Two batches were carried out for each starch.

Bread Characterization: Moisture was determined in two steps according to the AACC method 44-15.02.[32] The analysis was performed in duplicate.

Crumbs color and texture of three breads from each starch were analyzed. The texture of the crumb of breads made with non-traditional starches was compared to that of cassava bread. The crust was carefully removed with a scalpel, and the texture of 1 cm thickness crumb cubes of 2 cm x 2 cm was determined by a double compression test using a Texture Exponent TA.XT.PLUS equipment (Stable Microsystems, Surrey, UK) with a P36 aluminum cylindrical probe. The crumb was compressed to 50% of its original height at a crosshead speed of 1 mm s^{-1}, with a 30 s interval between compressions. Color parameters (L^*, a^*, and b^*) of the crumbs were measured using a CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). Three color measurements were made on each slice.

Slices were scanned with a resolution of 600 dpi, and the slice area and the alveolar density were calculated using the ImageJ software v. 1.53K and following method described by Espinosa-Ramírez et al.[34] Bread volume was estimated from the slice area and the depth of the mold (3 cm). Cell size was calculated as the average area of the alveoli in the analyzed section, and cell density was calculated as the number of alveoli divided by the analyzed area, expressed as alveoli cm^{-2}.

Statistical Analyses: Statistical analyses were performed using the Infostat software (v2011). Data were analyzed by a one-way ANOVA followed by a Least Significant Difference (LSD) test at $p = 0.05$. A correlation matrix was built considering all the physicochemical, thermal, and pasting properties of the starches and the volume increase of the fermented batter, the volume of the breads, and their texture parameters using Statgraphics CenturionXVII software (Bitstream, Cambridge, MA, USA). Pairs of data with Pearson correlation values (r) above 0.9 which resulted significant at $p < 0.05$ were considered as correlated.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

C.D.: Investigation; writing – original draft; data curation; formal analysis; R.G.: Conceptualization; supervision; formal analysis; writing – review & editing; C.M.R.: Conceptualization; methodology; funding acquisition; investigation; writing – review & editing.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

amylose, bread, DSC, hydrolysis, viscosity

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