Original article[1]

Chuño prepared experimentally from three varieties of Bolivian potatoes (*Solanum spp.*): starch extraction, amylose quantification, and microscopy imaging

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Keys: *Chuño - "sun-exposed-freeze-drying potato", Starch, Potato, Variety, Extraction, Amylose, Microscopy.*; **Claves:** *Chuño – "papa deshidratada en frío y al sol", Almidón, Papa, Variedades, Extracción, Amilosa, Microscopía.*

ABSTRACT

Chuño, prepared experimentally from three varieties of Bolivian potatoes (Solanum spp.): starch extraction, amylose quantification, and microscopy imaging . Chuño was obtained from three potato varieties, Luki, Imilla, and Holandesa, through a sun-exposed-freeze-drying potato process with a 70-80% weight loss due to water removal. Starch extraction yield was 49% in potato and 30% in chuños as average. Different amylose contents depending on the chuño variety were obtained. Luki showed a lower output. in the range 30.87% to 28.76%, whereas that Imilla had the highest levels in both chuño and potato (37.12% and 33.61% respectively). This could contribute to the existence of retrograded starch. Starch granules showed elliptical and spherical shapes in both potato and chuño. However, chuño granules were more elongated, irregular, and compact, which could reduce the water absorption capacity.

RESUMEN

Chuño preparado experimentalmente a partir de tres variedades de papa boliviana (Solanum spp.): extracción de almidón, cuantificación de amilosa e imágenes de microscopía. Se obtuvo chuño de tres variedades de papa, Luki, Imilla y Holandesa, mediante un proceso de deshidratación en frío con una pérdida de peso del 70-80% debido a la eliminación de agua. El rendimiento de extracción de almidón fue del 49% en papa y del 30% en chuños como promedio. Se obtuvieron diferentes contenidos de amilosa dependiendo de la variedad de chuño. Luki mostró un rendimiento menor 30.87% y 28.76%, mientras que Imilla tuvo los niveles más altos tanto en chuño como en papa (37.12% y 33.61% respectivamente). Esto podría contribuir a la existencia de almidón retrógrado. Los gránulos de almidón mostraron formas elípticas y esféricas tanto en papa como en chuño. Los gránulos de chuño fueron más alargados, irregulares y compactos, lo que podría reducir la capacidad de absorción de agua.

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INTRODUCTION

Potato starch obtained from the tubers of *Solanum tuberosum* is an important polysaccharide that serves as a source of carbohydrates in the human diet and has multiple applications in industry. Its importance is underlined by its physicochemical properties, which include high swelling power, transparency and digestibility, making it suitable for use in food products, pharmaceuticals and industrial applications[1 2 3]. The starch content in potato tubers can vary from 10% to 25% of their fresh weight[2 3].

In the Andean region of South America, one of the ancestral ways of preserving potatoes is to obtain the product called "chuño"[4]. In the chuño production process, a high percentage of moisture is eliminated from the potato tissue due to the fluctuating temperatures during the Andean winter season[4], a period in which temperature variability is 0 to -13.5 ºC at night and 16 ºC to 4 ºC during the day[5]. The moisture reduction process is due to cell lysis in the tuber structure during the freezing and thawing cycles, which allows the removal of water by mechanical pressing. At the same time, low temperature sublimation is possible, since the ambient relative humidity is low, between 30 and 40%[4].

Over the years, chuño has been made from the so-called "bitter potatoes", which include the varieties Sutumari (*Solanum tuberosum* ssp. andigena), Qita Luki, Lloqallitu, Saqampaya, Pocotoro and Luki[6]. However, chuño is currently produced from other potato varieties under the criterion that they cannot enter the market as common potatoes.

In the bibliographic review on chuño carried out in 2021[7], focused mainly on starch, it is concluded that there is great evidence of structural changes in starch resulting from the transformation of potato to chuño due to the influence of UV radiation and drastic temperature changes. These conditions could lead to the activation of enzymes, for the catalysis of certain reactions such as the breaking of bonds, which would lead to physicochemical changes in starch.

EXPERIMENTAL

Three varieties of potato were used: Luki Turno (*Solanum x juzepczukii*, a common variety in the production of chuño, or bitter potato) and two other varieties, Condor Imilla *(Solanum tuberosum ssp. andigena*), and Holandesa (*Solanum tuberosum*), not explicitly used in the production of chuño, but important due to its consumption.

Potato starch extraction

The process used for potato starch extraction is semi-industrial[8] with modifications[9]. The method involves the following steps: washing and peeling of potato samples, cutting into 1 cm thick cubes, placing samples in sodium bisulfite solution (0.1% w/w) at a 1:1 (g/ml) ratio and blending at high speed for 2 minutes. Sieving (140 µm) of the solution and obtaining "starch milk". Refrigeration (4°C) for 4 hours and decanting. Successive stages of suspension in cold water (4°C, 1:1 g/ml ratio) for 15 min, and decanting. Finally, suspension in ethanol (95%), decanting (1:05 g/ml ratio) and drying at room temperature.

Obtaining of chuño and tunta

In the Bolivian and Peruvian highlands, two products are obtained from potatoes, each with different stages and therefore different final characteristics[10], "chuño or black chuño", the subject of this study, and "tunta or white chuño".

The tunta obtaining process involves the prolonged exposure of the tubers to water followed by freezing in the open air by night frost (below zero); they are collected at dawn, avoiding blackening due to daytime exposure to the sun.[10 11 12]. After several nights of freezing, the potatoes are placed in sacks and submerged in lagoon water or slightly salty running water where they will acquire the softness characteristic of a superior quality[13]. Black chuño obtaining is not by soaking potatoes in water but of exposing it to the sun, freezing (open air overnight below 0°C), pressing and drying it; although the process may have certain regional variations[10 11 12 13]. The characteristic black color of black chuño comes from exposure to sunlight over a period of several days[14]. The production of chuño from the three varieties of potato emploted in the present work was carried out following the process shown in Figure 1.

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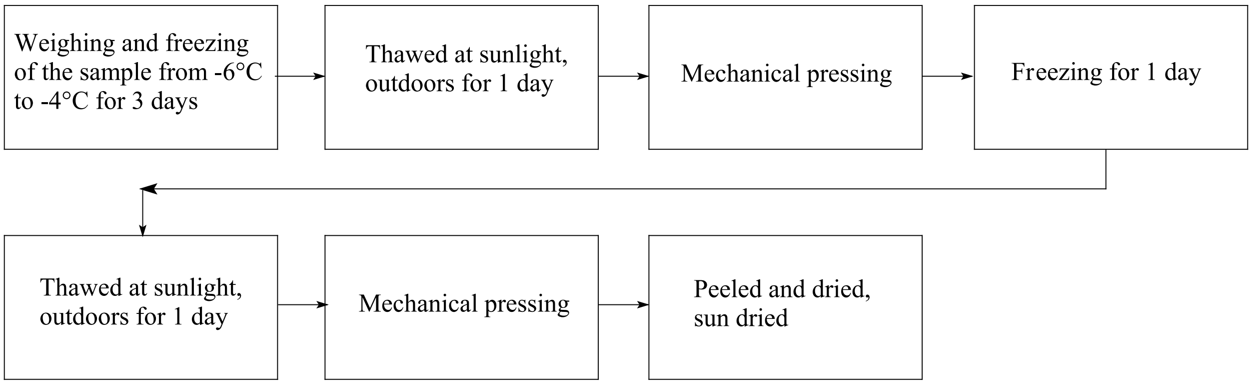

Figure 1. General diagram of the production of chuño from potatoes. The first three steps are repeated in cycles of 3 to 6 times depending on the size and/or variety of the sample

The percentage of weight loss was quantified twice per cycle: after the freezing stage and after the pressing stage. For this procedure, three samples of each variety were selected, weighing between 64 g and 100 g to ensure a similar range of initial weights. This yielded the percentage of weight loss for each cycle and the percentage of total weight loss resulting from the chuño production process.

Extraction and quantification of chuño starch
A modified potato starch extraction method was employed (see above). The first two steps were modified in which the chuño was weighed and placed in distilled water in a 1:3 w/w ratio for 48 hours. The hydrated chuño was cut into cubes approximately 1 cm thick and placed in water in a 1:2 w/w ratio. The process described above was continued, from the "high-speed blending" stage.

Amylose quantification
We replicated ISO-1987 standard method for potato and chuño samples[15 16].
The absorbance of the iodine-amylose complex was measured using a UV-Vis spectrophotometer Biotek Instruments, Inc. Winooski, USA at a wavelength of 620 nm. The percentage of amylose in the potato starch samples was obtained using a calibration curve with external standard amylose standards:

1. Amylose, Type III: From potato essentially free of amylopectin (Sigma Aldrich, Chemie Gmbh, Steinheim, Germany)
2. Amylopectin, from potato starch (Sigma Aldrich, ChemieGmbh, Germany)

The calibration curve was 0%, 10%, 25%, 30%, 40% amylose and sodium hydroxide (1M), Ethanol (95%), sodium hydroxide (0.09M), acetic acid (1M) and Lugol's (2%) solutions.

The direct percentage of amylose obtained from the absorbance and the calibration curve was adjusted accordingly by subtracting the moisture quantified in the starch with a moisture balance Radwag MAC 110/WH.

Starch morphology
For morphological characterization, an optical microscope Optika Vision Pro (M.A.D. Apparecchiature Scientifiche S.R.L., Ponteranica, Italy) with a built-in USB digital camera was used and the shape of the observed starch granules was morphologically described.

RESULTS AND DISCUSSION

Obtaining chuño from potatoes
Process for obtaining chuño from the three varieties of potatoes used: Imilla, Luki, and Holandesa, see Figures 2, 3 and 4.

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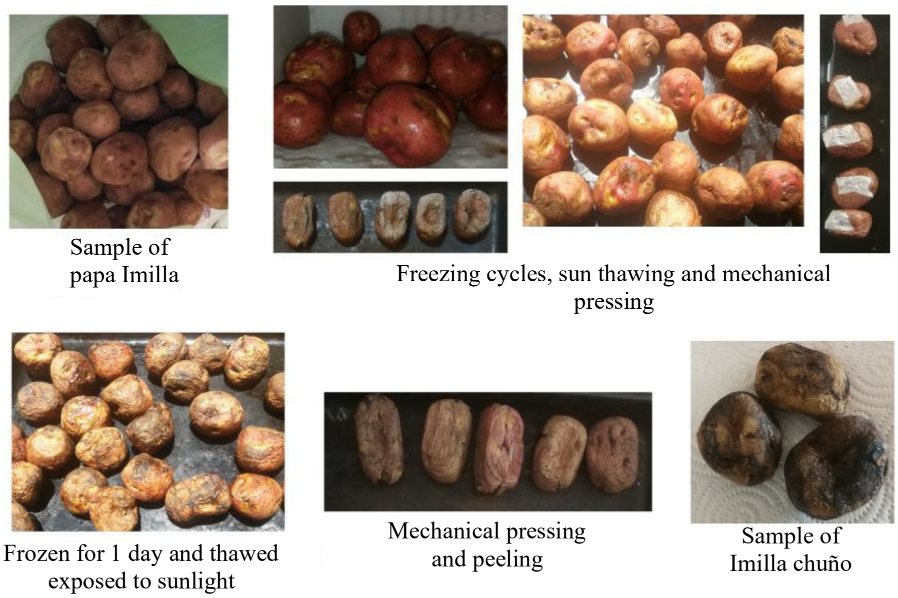

Figure 1. Images of the process of obtaining chuño from potatoes – Imilla variety (photos by F. V.)

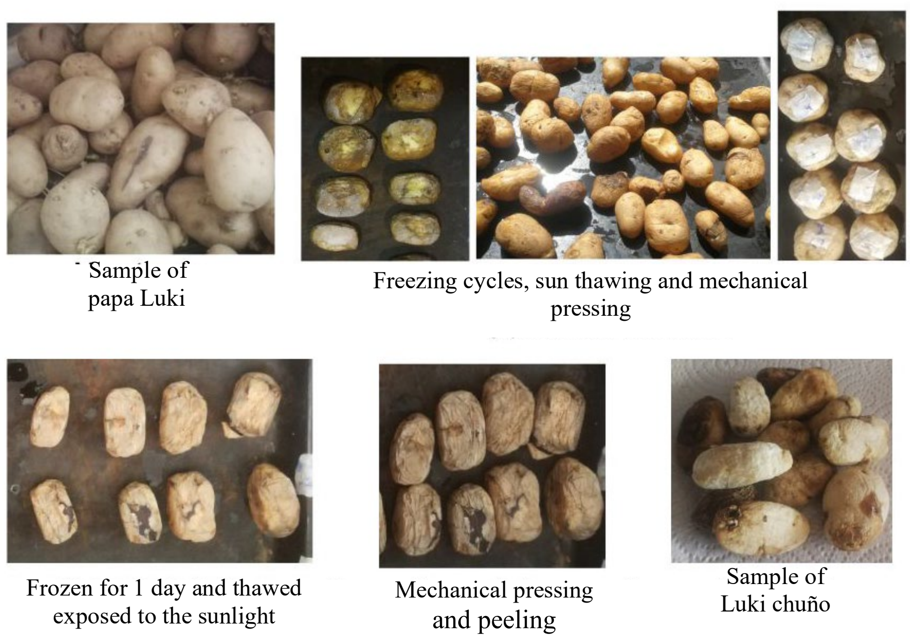

Figure 2. Images of the process of obtaining chuño from potatoes – Luki variety (photos by F. V.).

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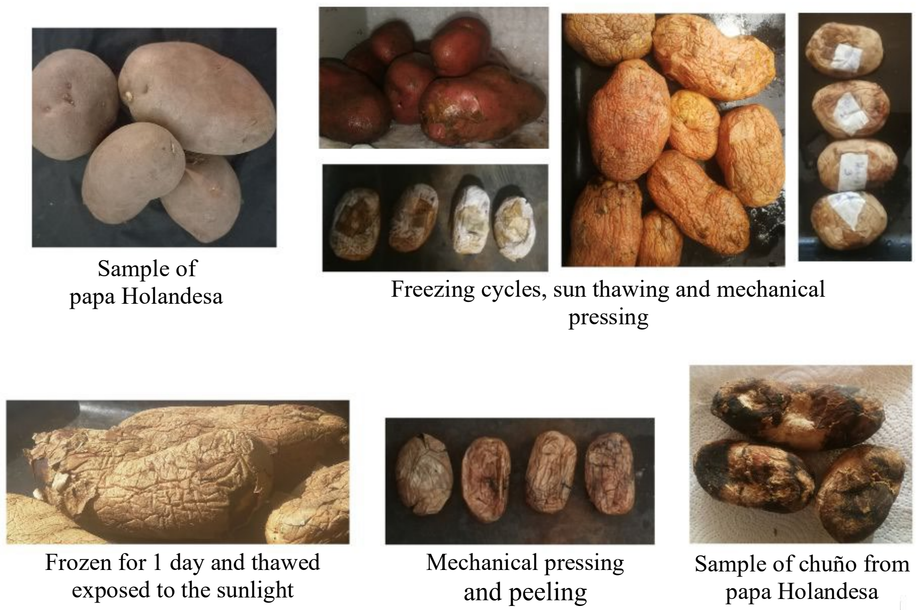


Figure 3. Images of the process of obtaining chuño from potatoes – Holandesa variety (photos by F. V.)

In the chuño production process, the freezing stage causes the water present in the potato to change phase from liquid to solid. This transformation increases the volume of water due to the expansion that occurs upon freezing, causing cell lysis. This lysis causes the rupture of cell membranes and the dissociation of water molecules from other structures present in the potato's plant tissue[4].

During the day the sample thaws and the water changes from solid to liquid (at typical winter temperatures in the Altiplano of 16 ºC to 4 ºC)[5]. As a result of the structural changes generated during the freeze/thaw cycle and mechanical pressing, the elimination of water along with other compounds is facilitated. The lost water contains phenolic compounds and organic acids[4].

The texture of the final product, after final drying and peeling, is completely different from that of a potato. It is a solid product with varying colors depending on the variety and much lighter than the initial sample.

Three samples per variety were selected to calculate the percentage of weight loss during the chuño production process (see Figure 5).

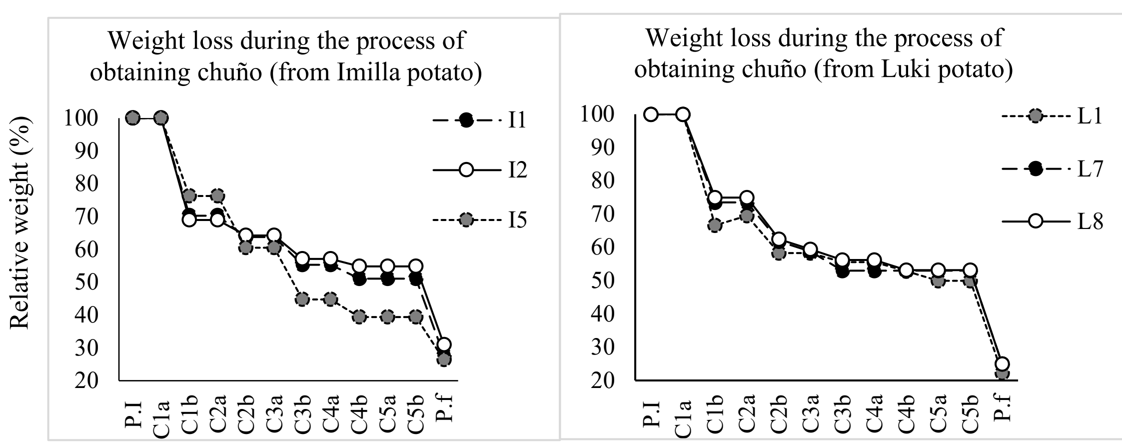
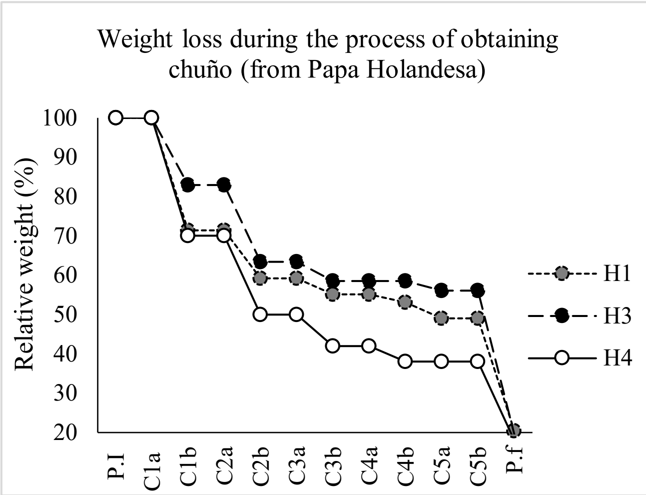


Figure 5. Percentage of weight loss in the chuño production process – Imilla, Luki and Holandesa varieties



P-I = initial weight, C1 = first cycle, C2 = second cycle, C3 = third cycle, C4 = fourth cycle, C5 = fifth cycle, a = weight after freezing, b = weight after mechanical pressing, P.f = final weight. I1, I2, I5 = identifiers of the selected samples of imilla potato. L1, L7, L8 = identifiers of the selected samples of luki potato. H1, H3, H4 = identifiers of the selected samples of Papa Holandesa.

Figure 5. *Cont.* Percentage of weight loss in the chuño production process – Imilla, Luki and Holandesa varieties

These results (Figure 5) show weight loss during the chuño production process: 71.7% for the Imilla variety, 75.9% for the Luki variety, and 80.7% for the Holandesa variety. Since similar initial weights were selected, the percentage loss varies between 70 and 80%, with differences related to the composition and structural characteristics of each variety.

Additionally, it is observed that the most evident change occurs during the first cycle, after mechanical pressing. The first cycle involves the greatest amount of water removal. On the other hand, after the last cycle and obtaining the final weight, the loss of skin and complete drying are observed, resulting in samples with weights less than half the weight of the potatoes.

In subsequent analyses, the final percentage of the weight of chuño samples will be considered as the existing percentage of dry matter of each potato variety.

Extraction and quantification of starch from Potato and Chuño Samples

Table 1 shows the starch extraction yields for each variety of potato and chuño samples.

Table 1. Starch extraction yield - potato samples.

	Potato			Chuño
Variety	**Total yield (%)**	**Dry sample (%)**	**Dry weight yield (%)**	**Yield (%)**
Imilla	10	28	35	29
Luki	15	24	62	38
Holandesa	10	19	51	23

Total yield = starch extraction yield relative to the total potato samples
Dry sample = percentage of dry matter in the potato samples
Dry weight yield = starch extraction yield relative to the percentage of dry matter in the potato samples
Yield = Yield relative to the total chuño samples

In the case of potatoes, the percentage of dry sample was obtained from the data on the percentage of final weight of chuño. In this way, the approximate percentage of water present in the potato samples is obtained (76%), which is close to the normal values from the literature which reported the potato with around 20% dry matter[17 18]. The extraction yield - dry weight yield was calculated by using the percentage of dry sample.

According to Table 1, Luki and Holandesa varieties exhibited a relatively high starch extraction yield, 62% and 51% respectively. The Imilla variety starch extraction yield was lower (35%). This is possibly due to structural differences in the tuber composition.

Comparison of the starch extraction yield of chuño and potato shows the double in chuños than potatoes. Chuño is a product composed primarily of dry matter, so a comparison with the dry weight starch extraction yield from potatoes is consistent. Ratios of 1:0.8, 1:0.6, and 1:0.4 are observed in yields for potato starch vs. chuño starch extraction for the Imilla, Luki, and Holandesa varieties, respectively (Table 1).

In comparison, the extraction yield in potato is higher than the extraction yield of chuño, possibly for the following reasons: in the process of extracting starch from chuño, described in the experimental part, it starts from an initial step of hydration of the sample where it is possible that there is a loss of starch. Additionally, it is possible that in the process of obtaining chuño from potato there is incidence of UV light that can affect the structure of starch and break certain bonds[19 20], facilitating the subsequent loss of starch or in another case, creating new bonds with other molecules that hinders the extraction of starch. Finally, there may be a minimum loss of starch in the process of freezing, drying and mechanical pressing of potato to chuño.

The Luki potato was the variety with the highest extraction yield in all cases. This can only be a consequence of specific characteristics of this variety, such as the percentages of the present components including starch.

Quantification of the amylose in starch

Table 2 shows the percentage of amylose in the starch of each potato and chuño variety. These data were corrected for moisture as indicated in the experimental section.

Table 2. Percentage of amylose in the starch samples.

Variety	Amylose Potato Samples (%)	Amylose Chuño samples (%)	Amylose loss(%)
Imilla	37.12	33.61	9.45
Luki	30.87	28.76	6.86
Holandesa	32.61	25.14	22.90

The amylose values of the Luki and Dutch potato varieties are close to the range normally reported, 20-30% of the starch[21] and close to that reported in studies of potatoes of similar origin whose value was 27.03 + 3.59[22].

The highest value 37.12% for Imilla potato shows different characteristics regarding other varieties. Differences in amylose content between different potato cultivars, even of the same variety, can be attributed to environmental factors, indicating variability in the reported values due to growing conditions[23].

During the chuño production process a decrease in amylose from the total starch content was proven, meaning by this the modification of the starch structure. However, this decrease didn't exceed 10% in the three potato varieties traditionally used for chuño production, specially the Luki potato. Therefore, the different amylose/amylopectin ratio in chuño may depend on the potato variety employed in the process.

Starch content in potato and chuño is high on amylose in Imilla and Luki varieties, an indicator of resistant starch[24]. Amylose is known to have a propensity to form crystalline structures, which may have an impact on digestibility and functionality in food applications. Variations in potato starch can affect retrogradation, which directly correlates with amylose content, indicating its crucial role in starch behavior during cooking and cooling[25].

Samples' morphology

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Figure 6 shows the microscope images of potato and chuño starches.

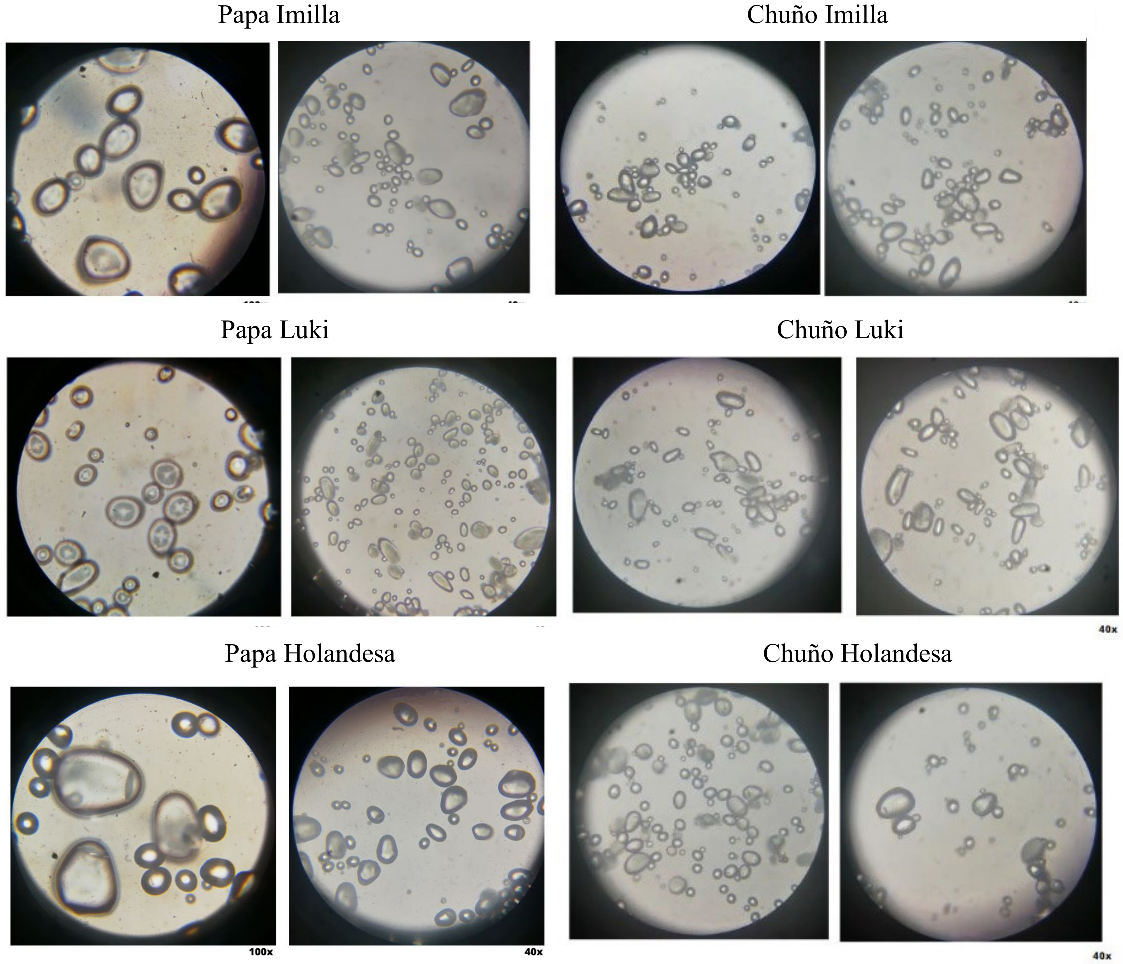

Figure 6. Microscope images of potato and chuño starch samples (microscopy photos by F.V.)

Potato starch granules are usually larger than those of rice, cassava, or quinoa[22]. The granules have an elliptical and spherical shape[16], with the larger granules usually having a more pronounced elliptical shape compared to the smaller granules. This morphology is observed in all varieties, both in potato and chuño.

In potato starch, the Imilla variety presents more irregularities in the larger granules than in the smaller ones. The Luki variety has no granules with visible irregularities and has smaller granules with an almost spherical shape. The Dutch variety has visibly larger granules, with greater irregularities as the granule size increases.

In chuño starch, most granules are more elongated and elliptical in shape. More irregular granules are also observed, especially in the Imilla and Luki varieties.

Due to the dehydration process to obtain chuño, starch granules may be smaller or more compact.

The shape and size of starch granules can drive the ability to absorb water and the conditions required for gelatinization as well as other characteristics such as amylose content or the presence of other components[26]. It could be inferred that larger elliptical granules may provide a greater surface area compared to more compact granules,

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potentially improving their water absorption properties. Therefore, it is possible that chuño starch has a lower water absorption capacity than potato starch.

CONCLUSION

The freeze-drying process of potatoes to obtain chuño produces a 70-80% weight loss due to the loss of water content. The average starch extraction yield is 49% for potatoes, with the Luki variety reaching 62%. For chuño, the average yield is 30%, with the Imilla variety being the most notable at 38%.

The amylose content of chuño is lower than that of potato starch, with a differentiated variation depending on the variety. The Luki variety shows a smaller reduction, from 30.87% to 28.76%, while Imilla has the highest levels in both chuño and potato; 37.12% in potato and 33.61% in chuño. Having high levels of amylose, these starches could be considered to have a high level of retrogradation[25], which is a type of resistant starch[7 27].

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