

# Comparative Morphology of Starch of Three Andean Tubers

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The starch of three Andean tubers have been studied (*Oxalis tuberosa*, *Ullucus tuberosus*, *Tropaeolum tuberosum*). Qualitative optical methods, scanning electron microscopy and quantitative analytical microscopy have been used. Statistical tests have been applied to the results. The comparative study of starch grains morphology provides important characters for their identification. As a result, starch would be an element of high value in the determination of material recovered from archaeological sites in different preservation conditions or used in food elaboration.

**Vergleichende Morphologie der Stärke von drei Anden-Wurzeln.** Die Stärke von drei Anden-Wurzeln wurde untersucht (*Oxalis tuberosa*, *Ullucus tuberosus*, *Tropaeolum tuberosum*). Hierzu wurden qualitative optische Methoden, Raster-Elektronenmikroskopie und quantitative analytische Mikroskopie angewendet. Auf die Resultate wurden statistische Tests angewendet. Die vergleichende Untersuchung der Stärkekorn-Morphologie erbrachte aichtige Charakteristika für ihre Identifizierung. Im Ergebnis ist die Stärke ein Element von hohem Wert in der Bestimmung von Material im archäologischen Bereich unter verschiedenen Präservationsbedingungen oder bei der Zubereitung von Lebensmitteln.

## 1 Introduction

Man employs for feeding several underground organs, rich in carbohydrates, domesticated in the beginning of agriculture. The species here considered are three microthermal tubers (named in that way by Parodi [1] because they resist very low temperatures): "oca" (*Oxalis tuberosa* Mol. Oxalidaceae), "ulluco" (*Ullucus tuberosus* Caldas – Basellaceae) and "añu" or "mashwa" (*Tropaeolum tuberosum* Ruiz et Pav.-Tropaeolaceae). These tubers are a very important source of food to the Andes inhabitants, from Venezuela to Northwestern Argentina. They are grown in small fields and terraces systems at altitudes between 2500–4250ms m [2].

Several institutions (FAO among them) are very interested in the preservation of biodiversity of these species because, besides agricultural characteristics mentioned earlier, they constitute highly energetic foods with a variable amount of proteins in different clones [3].

This paper undertakes to accomplish a comparative study of these tubers starches in order to distinguish with them the different species. These characters could be employed for identification of samples consisting of limited material or in different preservation conditions. It is well known the uses of starch grains as diagnostic characters [4–6]. On account of this, the methodology employed here is that which modifies in the least way the starch grain morphology and requires at the same time small amount of material [7].

### Oca (*Oxalis tuberosa*)

The oca is an endemic crop of the Andes. It seems to have been domesticated in Central Peru and Northern Bolivia where the major diversity of wild and cultivated forms are found. From there it spread in pre-Columbian times to Venezuela (8° lat. N) and North of Argentina and Chile (25° lat. S). Two or three hundred years ago its culture has been introduced in Mexico and last century in New Zealand, where its popularity increased in the last twenty years [8].

The tubers must be exposed to sun to develop a sweeter taste. After several days ocas may be eaten, either raw but more commonly boiled or roasted. They are also prepared like sweet potatoes. The sweet varieties are sometimes cut in strips and pla-

ced in the sun to form a product called "cavi", which is cooked slowly and afterwards eaten as a dessert with honey or sugarcane syrup [9]. The inhabitants of the High Andes store their tuber crops in a desiccated form called "chuño": the tubers are frozen and then dehydrated. In the case of oca, this preparation is called "khaya" [8] and the best oca "chuño" is the bitter variety which constitutes for this reason the greater part of the Peruvian crop [9].

The nutritional value of the tubers (presented on a dry weight basis) is very variable: 83.0–88.8% carbohydrates, 3.0–8.8% proteins and 0.5–0.6% fat. Moisture reaches 80.2–84.6% [3]. They contain a fair quantity of ascorbic acid [10].

Archaeological records of *Oxalis* are mentioned for the Guitarero Cave (Ancash, Central Andes of Peru) by Smith [11] and Lynch [12], some possibly wild, some cultivated, dating from about 10500bp to 7500bp [13]. Material from Ancón-Chillón in the Peruvian coast, dated to 3750–3850bp in the initial period, were recovered by Moseley [14].

### Ulluco o melloco (*Ullucus tuberosus*)

Ulluco is one of the oldest and most popular useful plants of the Andes. In certain areas, it is outranked as a crop plant only by the potato [9]. The area of its culture extends from Venezuela (10° lat. N) to Northwestern Argentina and Northeastern Chile (25° lat. S). There is no precise evidence of its exact region of domestication, but its wild ancestor (*Ullucus aborigineus*) [15] is found from Huamachuco in Peru to Bolivia and Northwestern Argentina [16].

These tubers have the same range of culinary uses as do potatoes and there are a large number of traditional recipes containing ullucos, like soups and stews. They can also be used for salads. Some varieties present a larger quantity of mucilage and so must be boiled before preparation [8]. As ullucos decay easily, they can be prepared as desiccated "chuño", which in this species is known as "lingli" in Peru. Hodge [9] mentions that in Quito tubers are sliced and prepared with vinegar in cucumber fashion.

The nutritional value of ulluco (presented on a dry weight basis) is also variable: 73.5–81.1% carbohydrates, 10.8–15.7% proteins and 0.1–1.4% fat. Moisture reaches 86.0–86.2% [3]. Tubers have a high content of vitamin C (23mg/100g) and a fair quantity of phosphorus and other minerals [10].

Archaeological remains have been described by Smith [11] from Guitarrero Cave dating 10500–7500bp and supposed to be “ulluco” but this has not yet been confirmed. Definite identification of “ulluco” has been made at Ancón-Chillón dated to 4000–3500bp [13].

### Añu o mashwa (*Tropaeolum tuberosum*)

It seems to have originated in Central Andes from where it spread in pre-Columbian times to Colombia (8° lat. N) and Northwestern Argentina and Chile (25° lat. S). At present it is cultivated from Colombia to Bolivia at 3000–4000m altitude. At Northwestern Argentina it is rarely cultivated as a food crop but it is grown as an ornamental plant.

Añu is a rustic crop and it is cultivated in poor soils without fertilizants or pesticides because of its nematicidal and insecticidal properties [8]. It is important in the nourishment of the inhabitants of the marginal rural areas of high Andes. It is eaten in different ways of preparation: roasted, parboiled, as “thayacha” (the tubers are exposed to frozen during the night and the next day they are eaten with sugarcane syrup). The pulp is watery (80%) and nutritional values (presented on a dry weight basis) are very variable, as in the other tubers: 69.7–79.5% carbohydrates, 6.9–15.7% proteins and 0.1–0.4% fat [3]. They present a high content of ascorbic acid [10].

Añu has not a very pleasant flavour, so it would be most extensively used as fodder [8]. The tubers are said to have anaphrodisiac properties and they were given to Inca soldiers [9]. At present it is a fact that testosterone levels are significantly reduced in male mouses feed with añu [8].

Towle [17] mentions depictions of añu on Pacheco vessels dated at circa AD 1000, which are also cited by Yaccovleff and

Herrera [18]. No archaeological remains of añu seem to have been reported [13].

## 2 Materials and Methods

### 2.1 Materials

#### *Oxalis tuberosa*

**BOLIVIA:** No references about procedence, “caya” (chuño) (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); no references about procedence,

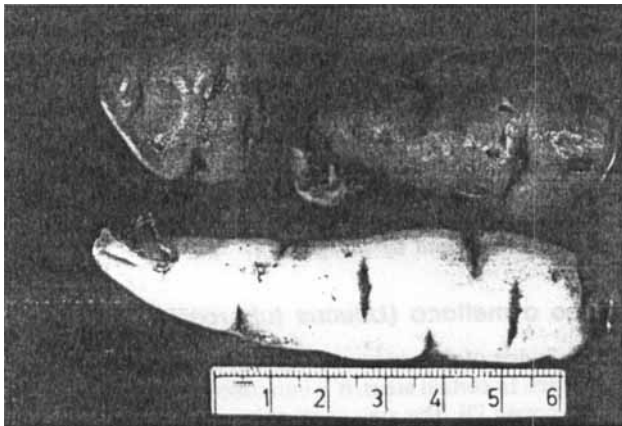


Fig. 1. Tubers of *Tropaeolum tuberosum*.

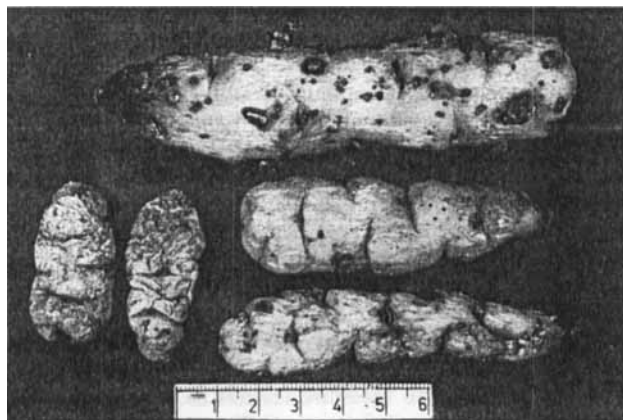


Fig. 2. Tubers of *oxalis tuberosa*.

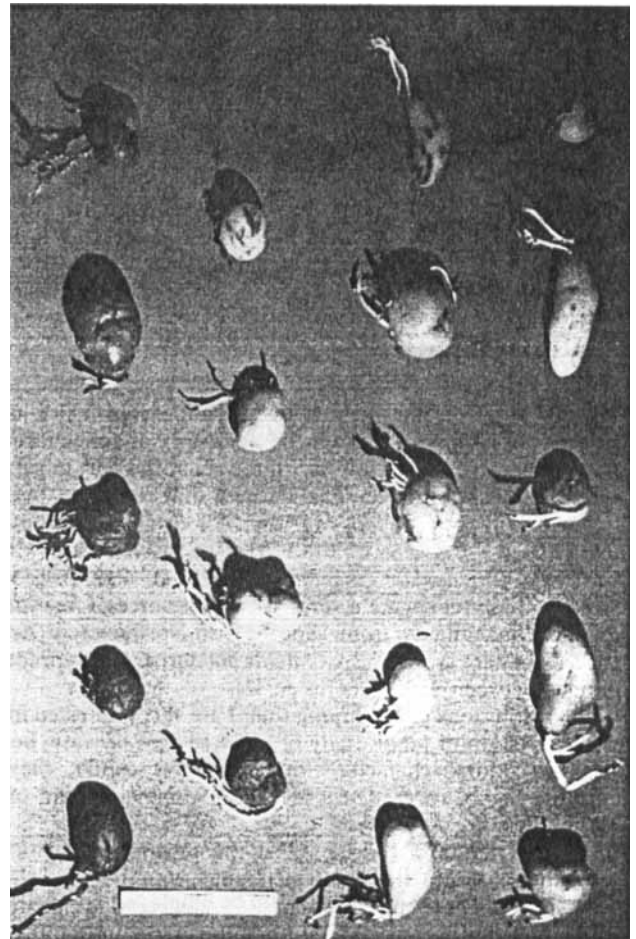


Fig. 3. Tubers of *Ullucus tuberosus*.

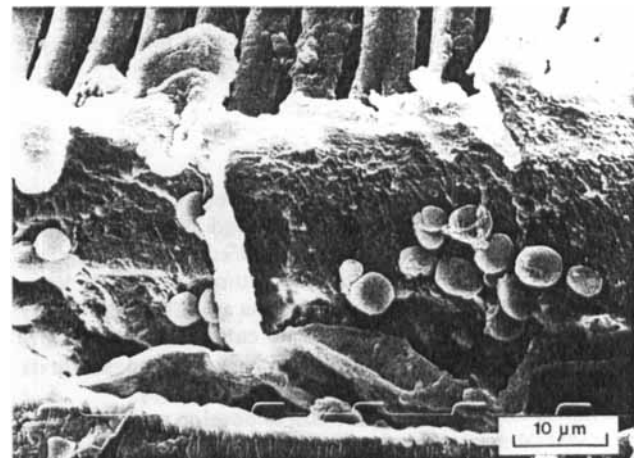


Fig. 4. Starch grains (less than 7µm) near the vessels, *Oxalis tuberosa* (SEM).

leg. *M. Balán*, "oca zapilla" (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); La Paz, Mercado, leg. *D. Delfino*, IX/1992 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). **ARGENTINA:** Jujuy: Dep. Capital, San Salvador de Jujuy, leg. *V. García Páez s/n*, VII/1992 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); Mercado de Abasto, leg. *M.L. Pochettino s/n*, 22/5/1984 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); Feria del Condimento, leg. *M.L. Pochettino* 499, 17/11/1993 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de la Plata). Dep. Humahuaca, Humahuaca, Feria, leg. *M.L. Pochettino s/n*, 29/4/1981 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Dep. Tilcara, Tilcara, Jardín Botánica de Altura, leg. *E.M. Zardini*, 29/4/1981 (LP). Salta: Dep. Capital, Salta, Mercado Municipal, 6/10/1981 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

**Ullucus tuberosus**

**ECUADOR:** Riobamba, Machala, Mercado, leg. *K. Saikkonen*, 1987 (Useful plants collection, LEBA, Facultad de ciencias Naturales y Museo de La Plata).

**PERU:** Cusco, mercado, leg. *R. Kalliola & J. Salo*, 1983 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Huancayo, Huapaipampa, mercado, leg. *P. Jokela et al.*, III-V/1987 (Useful plants collection, LEBA,

Facultad de Ciencias Naturales y Museo de La Plata). Pisak, Mercado (from Maskya) (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Puno, mercado, leg. *K. Saikkonen & J. Wright*, 1985 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

**BOLIVIA:** La Paz, mercado, leg. *D. Delfino*, IX/1992 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); La Paz, Los Andes, Catacora, campo Antonio Mamani, 3900m, leg. *P. Jokela et al.*, III-V/1987 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Dep. Potosi, Chulpa Kasa, campo Prudencio Apagani, 3600m, leg. *P. Jokela et al.*, III-V/1987 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Oruro, mercado, leg. *K. Saikkonen & J. Wright*, 1985 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Villazón, mercado (from Tarija), leg. *P. Jokela et al.*, III-V/1987 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

**ARGENTINA:** Jujuy: Dep. Capital, San Salvador de Jujuy, leg. *V. García Páez s/n*, VII/1992 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata); leg. *M.L. Pochettino* 500, 17/11/1993 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Dep. Humahuaca, Humahuaca, Feria, leg. *M.L. Pochettino s/n*, 29/4/1981 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata). Salta: Dep. Santa Victoria,

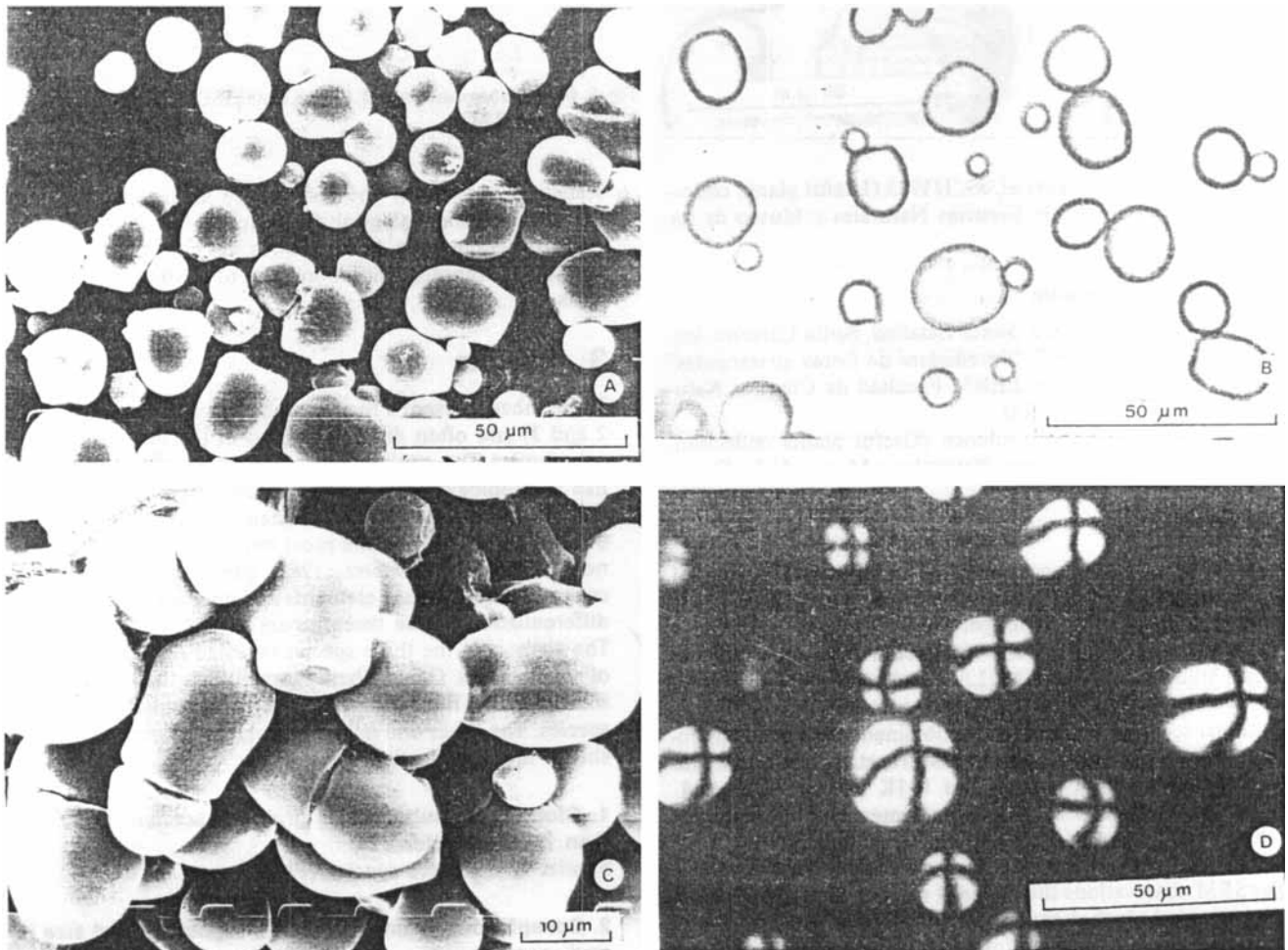


Fig. 5. *Tropaeolum tuberosum*: A. Single grains (SEM). B. Single grains (LM). C. Compound grains (SEM). D. Single grains (polarization microscope).

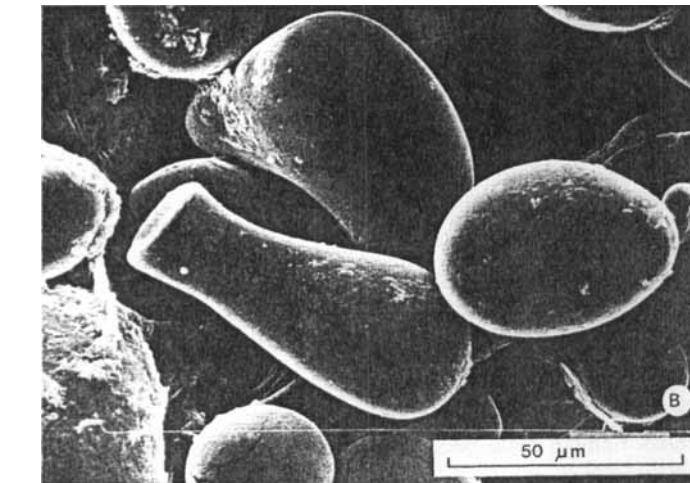
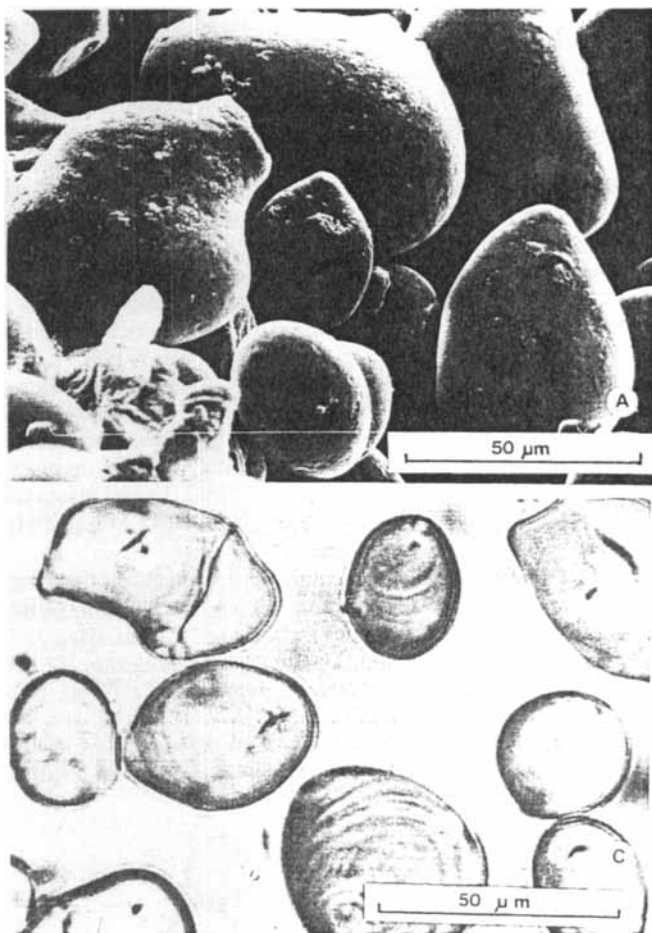


Fig. 6. *Oxalis tuberosa*. A and B. Single grains (SEM). C. Single and compound grains (LM).

San Isidro, leg. Zardini et al. 89, I/1983 (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

#### **Tropaeolum tuberosum**

**ARGENTINA:** Jujuy: Dep. Santa Catalina, Santa Catalina, leg. N. Vignale s/n, VII/1992, "enredadera de flores anaranjadas" (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

No references about precedence (Useful plants collection, LEBA, Facultad de Ciencias Naturales y Museo de La Plata).

### **2.2 Methods**

In order to eliminate accompanying impurities, the material was washed and soaked in water before its study. Then, it was studied fresh as well as dehydrated.

Starch grains morphology was observed with an optical microscope Mikro Rossbach Mod. LAC and microscope provided with polarized light Microlux Iroscope, in transversal and longitudinal sections of about 30µm, obtained with a hand microtome. Starch was also studied in disaggregation of parenchyma by scraping it with a needle. The I<sub>2</sub>-IK test was also used. From a microscope provided with a camera lucida, the microscopical pictures of the starch grains were transferred to a paper in order to obtain the corresponding measurements.

For SEM observations the material was dehydrated through a gradual ethyl alcohol series, cut in different directions and mounted in the corresponding stubs using a stereoscopic microscope. Preparations were coated with Au/Pd and photographed with a SEM Jeol JSM-T 100.

Statistical analysis of the result were applied to determine quantitative morphological differences. Material of each species proceeding from different sites was compared to discard possible morphology variations due to environmental conditions.

### **3 Results**

These tubers present a high morphological variability (Figs. 1, 2 and 3) and often different clones of the three species are very similar. This similitude increases in dehydrated material, like archaeological remains. Therefore, it is necessary to employ analytical microscopy for their identification.

Starch grain constitute the most important microscopic diagnostic feature. Melchiorre (1985) considers also epidermic cells, stomata and vase elements as valuable characters in the differentiation of the three tubers.

The analysis of the three species revealed two different types of starch grains. One of them is small (less than 7µm) and inconspicuous (Fig. 4) and they are present in all the studied species. The other one is highly variable in size and shape as shown in the following key.

#### **1. Globular or subglobular grains, inconspicuous, less than 7µm diameter.**

Similar in the three species (Fig. 4).

#### **2. Conspicuous grains, variable morphology and size for each species.**

A. Grains generally symmetrical, single: globular, subglobular or truncated (as the result of the desintegration of

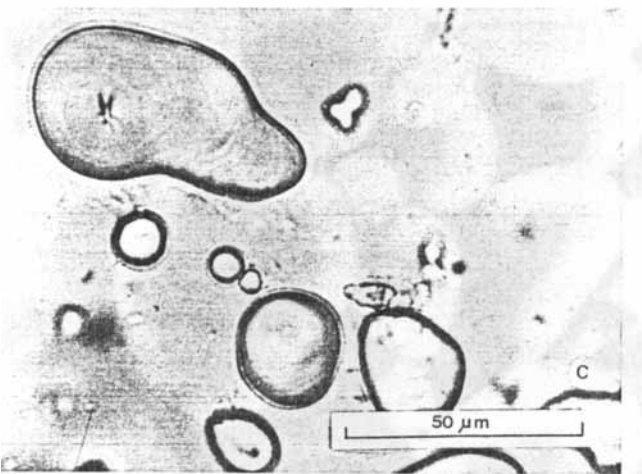
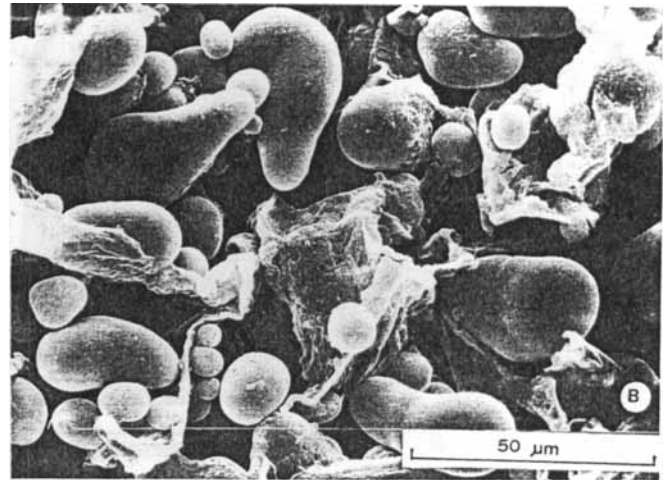
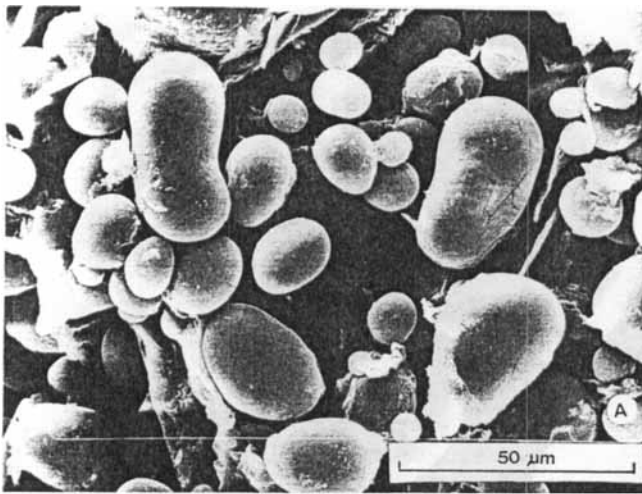


Fig. 7. *Ullucus tuberosus*: A and B. Single grains (SEM). C. Single grains (LM).

compound grains) ( $\times=8,83\mu\text{m}$ ;  $S=5,03$ ) (Fig. 5A and B), and compound by 2–3 granules generally unequal, with at less a symmetry axis (Fig. 5C). Polarization cross with well definite dark arms intersecting at one point (Fig. 5D) *Tropaeolum tuberosum*.

- AA. Grains generally asymmetrical, single: irregular shapes (pear-shaped, conoidal, prismatic) (Fig. 6A and B and 7A and B), and compound by 2–5 granules, generally unequal
- B. Grains of  $10\text{--}50\mu\text{m}$  ( $\times=22,01\mu\text{m}$ ;  $S=12,83$ ), compound grains asymmetrical (Fig. 8A and B), polarization cross with irregular, broken dark arms intersecting at more than one point (Fig. 9A) *Oxalis tuberosa*.
- BB. Grains of  $10\text{--}30\mu\text{m}$  ( $\times=15,79\mu\text{m}$ ;  $S=9,62$ ), compound grains with at less a symmetry axis (Fig. 8C and D), polarization cross with sinuous, non-well definite dark arms intersecting at one point (Fig. 9B) *Ullucus tuberosus*.

As demonstrated in Fig. 10 the first type of starch is not valuable to identify the different species because all of them present a mode ca.  $6\mu\text{m}$ , but show another mode at different points over  $10\mu\text{m}$ . This proves that the second type of grains are those useful as diagnostic characters.

#### 4 Discussion and Conclusions

As it was mentioned, the botanical identification of samples constituted only by underground organs (as those obtained in markets or archaeological sites) is very difficult because of the great variability of each taxa as well as the high similitude between several clones of these species.

It is possible to conclude here that starch grains have revealed themselves as an important diagnostic feature in this identification because of their abundance, ubiquity and resistance to different preservation conditions or food elaboration techniques.

The single grains of more than  $7\mu\text{m}$ , variable in size and morphology, as well as the compound ones constitute the most significant diagnostic character, whereas the globular single grains of less than  $7\mu\text{m}$  are not important because of their similitude in the three species. Ugent et al. [19] refer only single grains for these taxa but the presence of compound grains in smaller numbers than the single ones was confirmed with LM, SEM and polarized light.

Añu can be qualitatively differentiated from the other two species by distinctive features of shape and size of the significant grains. In the case of oca and ulluco, statistic analysis and polarized light observations show a great usefulness because of the higher variability in their starch grains.

#### Acknowledgement

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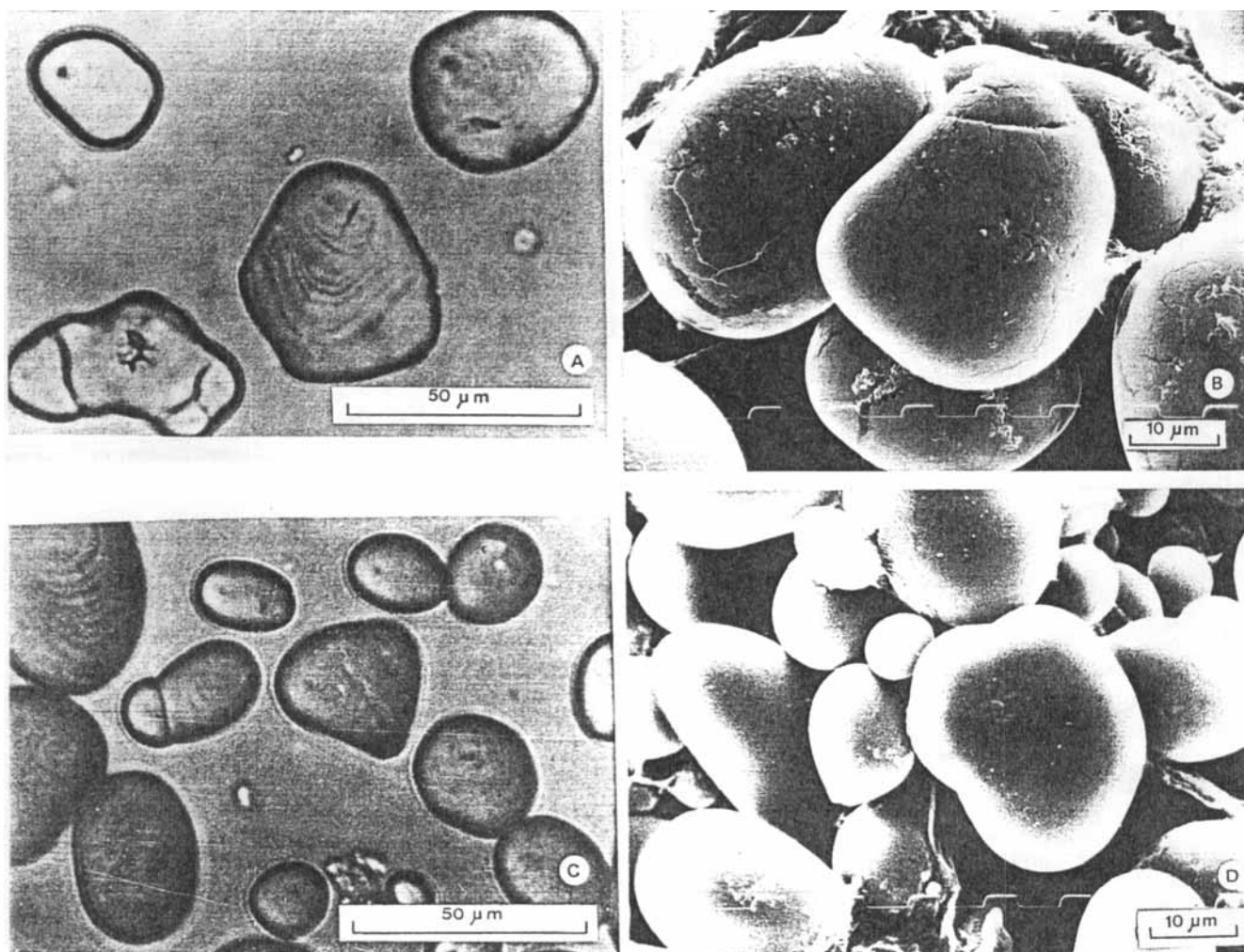


Fig. 8. Compound grains: A. *Oxalis tuberosa* (LM). B. *Oxalis tuberosa* (SEM). C. *Ullucus tuberosus* (LM). D. *Ullucus tuberosus* (SEM).

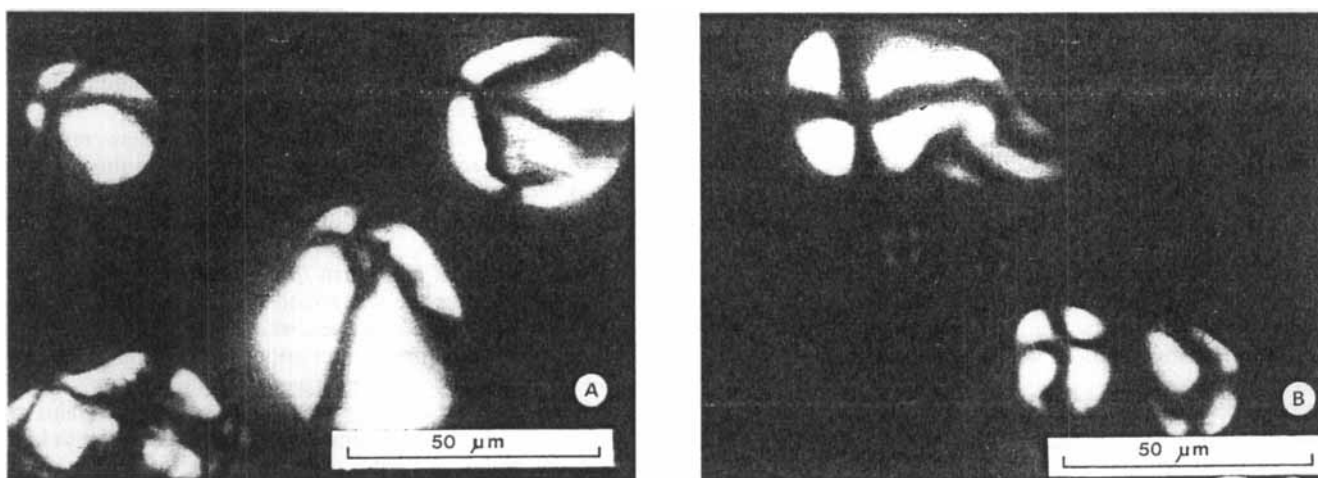


Fig. 9. Observation with polarization microscope: A. *Oxalis tuberosa*. B. *Ullucus tuberosus*.

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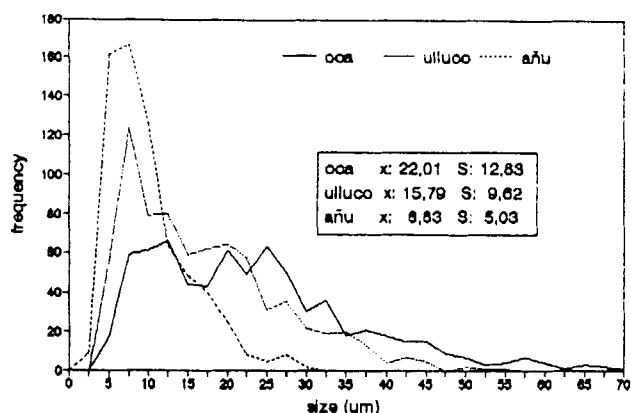


Fig. 10. Size distribution curves of starch from *Tropaeolum tuberosum*, *Oxalis tuberosa* and *Ullucus tuberosus*.

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# Thermal Behaviour of Calcium-Hydroxypropyl Rice Starch Complexes

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Thermal behaviour of hydroxypropyl rice starches and their calcium complexes were determined by using Differential Scanning Calorimetry (DSC) with starch:water in the ratio of 1:1. Glass transition and gelatinization temperatures were reduced by hydroxypropyl groups and enhanced by calcium treatment. Gelatinization enthalpies were reduced by both hydroxypropyl groups and calcium treatment. The interaction of hydroxypropyl groups and calcium with starch altered the two endothermic peaks observed in native starch into a single melting peaks.

**Das thermische Verhalten von Calciumhydroxypropyl-Reisstärke-Komplexen.** Das thermische Verhalten von Hydroxypropyl-Reisstärken und ihrer Calciumkomplexe wurde unter Anwendung der Differential-Raster-Calorimetrie (DSC) mit Stärke:Wasser im Verhältnis 1:1 bestimmt. Die Glasübergangs- und die Verkleisterungstemperaturen wurden sowohl Hydroxypropylgruppen reduziert als auch durch Calciumbehandlung verstärkt. Die Wechselwirkung von Hydroxypropylgruppen und Stärke und Calcium veränderten die beiden endothermen Peaks, die in nativer Stärke in einem einzigen Schmelzpeak beobachtet werden.

## 1 Introduction

Thermal behaviour of starch is important for evaluating and estimating the process design, unit operation, and quality of the final products. Abrupt changes in physical and functional properties of starches and starchy foods during heat processing leads to study of their thermal behaviour.

Thermal behaviour of starch granules are controlled by the granular size, amylose content, molecular weight, crystallinity, the internal granular organization and type and extent of

modification [1–3]. Solvents other than water also affect thermal behaviour of disrupting hydrogen bond within the granules, or by forming soluble complexes with the starch [3, 4]. Gelatinization temperature decreased with the increase of alkalinity and increased with the increase of salts of sodium upto a certain levels of concentrations [5, 6]. Young [7] observed that anions or cations caused a decrease or increase in starch gelatinization temperatures.

Hydroxypropylation lower the thermal transition properties and enthalpy associated with gelatinization [3, 8, 9]. The hy-