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ULTRASTRUCTURE OF QUINOA FRUIT (*Chenopodium quinoa* Willd)

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Abstract

The structure of quinoa (*Chenopodium quinoa*) fruit before and after germination was studied using electron microscopy. Protective coverings include a perianth consisting of loosely adhering cells which are readily removed by washing, a pericarp and two seed coat layers. Starch granules fill the perisperm cells and are arranged in 18 to 20 μm oblong aggregates. Limited hydrolysis of the starch occurs after 24 hrs of germination, with amylolytic erosion of large granules occurring at the hilum and periphery of the granules. Ungerminated embryo cells contain protein bodies with phosphorus-containing globoid inclusions. Essentially complete hydrolysis of the embryo protein bodies occurs within 24 hrs of germination leaving large central vacuoles within the cells.

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Key Words: Fruit structure; Quinoa starch; Germinated quinoa; Germinated seeds.

Introduction

Quinoa (*Chenopodium quinoa*, Willd) is a major staple food of people in the Andes. The fruit is used for porridges or ground into flour for preparing breads and cakes. Quinoa is a drought resistant crop and can be produced on land that will not support the growth of common cereals (White et al., 1955, Simmonds, 1965) so it is often used as a substitute for cereal grain in food preparations. The yield of fruit is 840 to 3000 kg/hectare (Simmonds, 1965).

The protein content of quinoa fruit ranges from 9 to 15% (Etchevers, 1980; Aguilar et al., 1979; Sanchez-Marroquin, 1983; Clavijo et al., 1973; Quiros-Perez and Elvehjem, 1957). Fat content is about 4% (Simmonds, 1965). Starch comprises about 60% of quinoa (Wolf et al., 1950). Quinoa starch has a diameter of 1-2.5 μm, a gelatinization temperature range of 57-64°C, an amylose content of 11%, and an average amylopectin chain length of 27 (Atwell et al., 1983). Quinoa starch pastes do not gel on standing (Wolf et al. 1950).

No reports have been published on the structural characteristics of quinoa other than a description of aggregated starch granules isolated from the fruit (Atwell et al., 1983). The anatomy of quinoa fruit is important from a processing standpoint as well as to increase our knowledge of its structural characteristics. The objective of this study was to describe the ultrastructure of quinoa fruit and the changes in structure as affected by germination.

Materials and Methods

Materials

Four quinoa varieties were studied: Blanca, Rosada, Pasankallo and Koito. The samples originated from Bolivia.

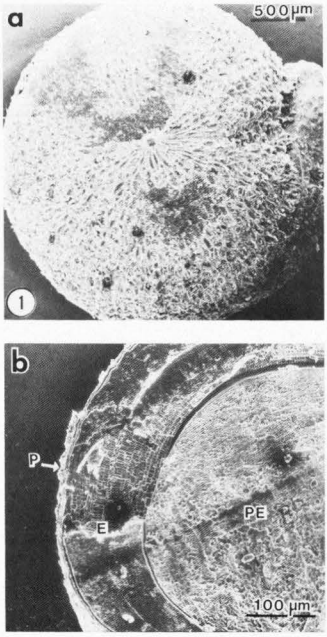
Methods

Scanning Electron Microscopy (SEM). The fruit was fractured with a dull razor blade, mounted on Al stubs, and coated with Au/Pd. Some samples were washed in distilled water and lightly abraded between the fingers to remove the loose outer layer of the fruit prior to SEM observations. Other samples were germinated by soaking in distilled water for 24 hrs. and then freeze-dried. Samples were viewed in an ETEC U-1 SEM operated at 10 to 20 kV or in a JEOL 35C.

Qualitative X-ray microanalysis of fractured, carbon coated samples was done using a Link X-ray Energy Dispersive Microanalyzer attached to a JEOL 35C SEM. Counting rates were 2500–3000 cs/sec and analysis time was 100 sec. at 20 kV.

Transmission Electron Microscopy (TEM). Some quinoa fruit were fixed for TEM following the simultaneous glutaraldehyde-OsO_4 fixation schedule of Franke et al. (1969). Other samples were fixed sequentially, first in glutaraldehyde and then in OsO_4 after buffer rinsing. Fixed samples were dehydrated in a graded acetone series (30% to 100%), embedded in Mascorro's resin (Mascorro et al., 1976), and sectioned with a glass knife on a Reichert OM-2 Ultra-microtome. Sections (60 nm thick) were stained with 5% uranyl acetate in 50% ethanol followed by lead citrate (Reynolds, 1963), and viewed in a Philips 201 transmission electron microscope at 60 kV.

Small fragments of moistened perisperm of ungerminated and germinated quinoa fruit were placed in copper specimen holders and frozen in liquid freon. Freeze-fracturing and Pt shadowing at an angle of 35° were done at −170°C in a Balzers Freeze Etch Unit. Replicas were cleaned by sonicating in Chlorox for 5 min., held in Chlorox for an additional 3 hr., washed in water, and collected on uncoated 300 mesh copper grids. Micrographs were taken on a Zeiss EM-10 transmission microscope at 60 kV.



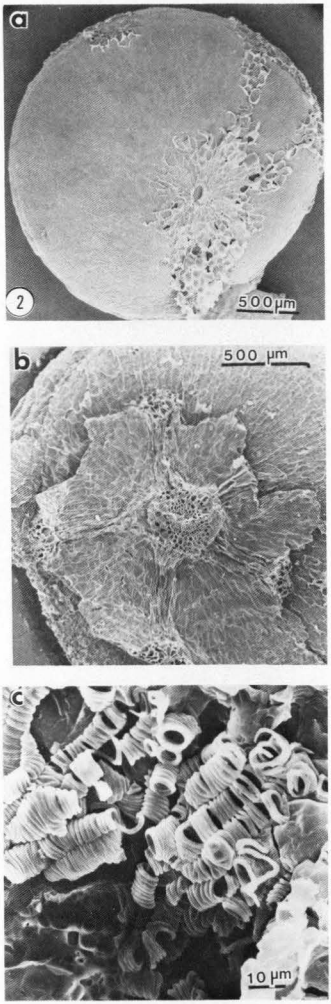


Figure 1. SEM of quinoa fruit: (a) and (b) enclosed in the perianth. Gross internal anatomy of quinoa shows pericarp and perianth (P), embryo (E), and perisperm (PE). Fracture done parallel to the plane of the cotyledon.

Figure 2. Scanning electron micrographs of quinoa: (a) perianth is removed by washing to reveal the pericarp, (b) the hilum, and (c) tracheids at the hilum.

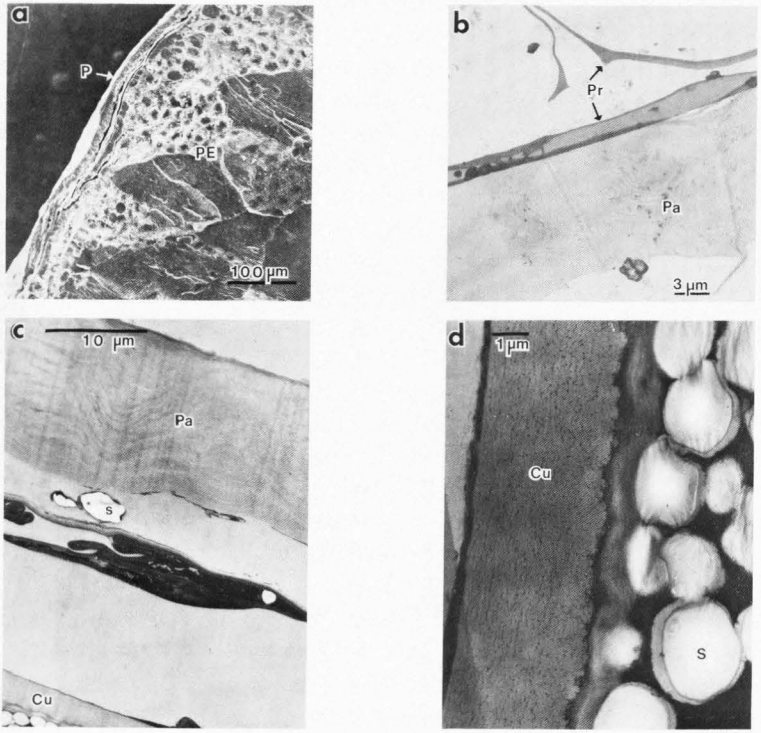


Figure 3. Quinoa covering layers. The location of the pericarp (P) and the perisperm (PE) in fractures perpendicular to the plane of the embryo is shown in the SEM micrographs (a). The pericarp (Pa) lies beneath the perianth (Pr) as seen in (b). TEM micrographs show starch granules (S) and electron dense structures in the seed coat layer beneath the pericarp (Pa) (c) and cuticle-like structure (Cu) is shown tightly attached to the perisperm (d).

Results and Discussion

Quinoa fruit are disc-shaped and range in diameter from 1 to 3 mm (Fig. 1a). The major anatomical parts of the fruit (Fig. 1b), the outer covering (perianth and seed coats), the perisperm, and the embryo, are described below.

Outer Coverings

Often when quinoa is harvested, the fruit fall off the plant still enclosed in the perianth (Fig. 1a). The weakly adhering cells of the perianth are easily removed by washing and scrubbing in water to expose the smooth surface of the pale yellow pericarp (Fig. 2a). The perianth of some varieties is magenta colored by a water soluble pigment that has a λmax of 530 nm which is characteristic of betacyanins (Harborne and Simmonds, 1964).

The hilum, the scar left from the attachment of the fruit to the placenta, is located at the center of the fruit (Fig. 2b). Tracheid structures involved in the transport of water and nutrients from the plant to the fruit can often be seen at the hilum (Fig. 2c).

Fractures perpendicular to the plane of the cotyledons (perpendicular to the disc structure, Fig. 3a) reveal starchy perisperm covered by a pericarp and seed coat structures. In this case, the perianth has been removed, but when present it consists of a layer of cells loosely attached to the pericarp (Fig. 3b). The pericarp layer consists of a dense, compact layer of cells about 10 μm thick (Fig. 3b and c). There are two seed coat layers beneath the pericarp. One layer is about 20 μm thick and contains polygonal starch granules and electron dense bodies (Fig. 3c). A second seed coat structure is cemented to the perisperm (Fig. 3d). This 3 μm thick structure may be the cuticle.

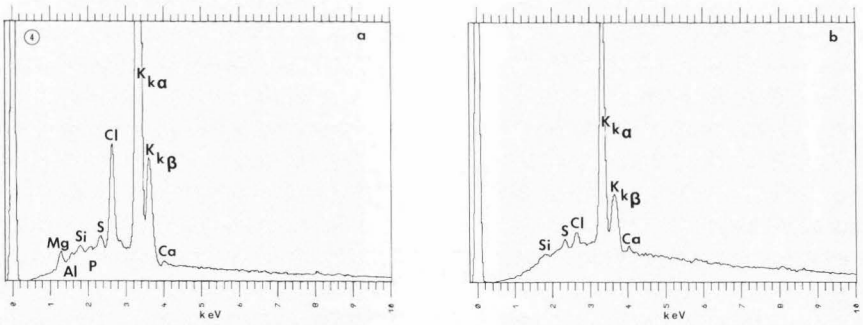
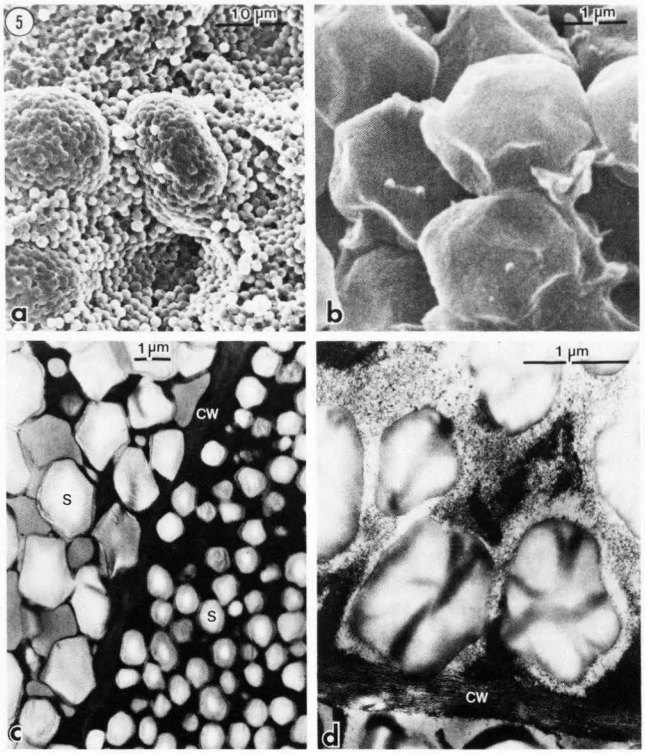


Figure 4. Qualitative X-ray energy dispersive analysis spectra of the perianth (a) and the pericarp after washing away the perianth (b). The Cu peak is from instrument parts. Both spectra printed at the 4 k vertical scale.



It is a common practice among quinoa consumers to wash and scrub the fruit in water prior to consumption (Simmonds, 1965). This washing removes the bitter saponins that are apparently located in the outer coverings (Aguilar et al., 1979). Washing removes the perianth which contains high K and Cl contents and minor or trace levels of Mg, Al, Si, P, S and Ca (Fig. 4a). It is doubtful that hand washing is vigorous enough to remove the pericarp. Like the perianth, unwashed pericarp contains high concentrations of K and similar low contents of S and Ca (Fig. 4b). However, there is less Cl in the pericarp than in the perianth.

Perisperm.

Quinoa fruit differs from cereal grains in that the storage reserves for the developing embryo are found in the perisperm rather than the endosperm (Wolf et al., 1950). The perisperm is located in the center of the fruit (Fig. 1b).

Starch granules in perisperm cells are polygonal and range in size from 0.4 to 2.0 μm (Fig. 5a and b). Transmission electron micrographs indicated that two populations of starch granule sizes exist in the perisperm: one population centers around a granule diameter of 0.5 μm; the other centers around a granule diameter of 1.3 μm (Fig. 5c). Atwell et al. (1983) reported a particle size distribution of 0.63 to 8.0 μm for a pure quinoa starch preparation with the median diameter being about 1.5 μm. Our data indicate that the size range is smaller and that a bimodal distribution exists. Some cells appear to contain only the larger granules while other cells contain mainly small granules (Fig. 5c). Starch granules are found as single entities within the cells (Figs. 5c and d) or compound structures consisting of spherical or oblong aggregates (Fig. 5a). As many as 14,000 starch granules may comprise an aggregate about 18 to 20 μm in size (Seidemann, 1966).

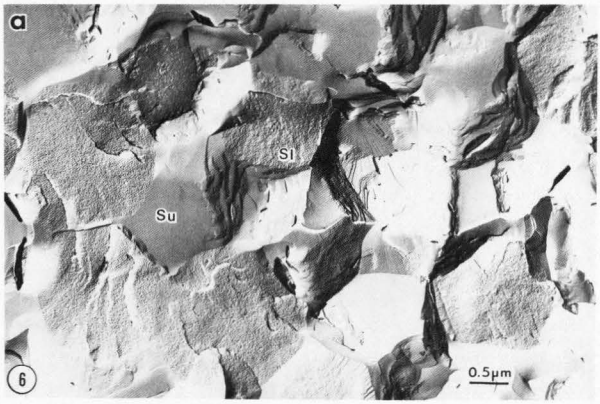


Figure 5. Quinoa perisperm cells. Aggregates within the cells (a) consist of polygonal starch granules (b). TEM micrographs (c and d) show cells with different size starch (S) granules. Granules are surrounded by matrix protein. CW – cell wall.

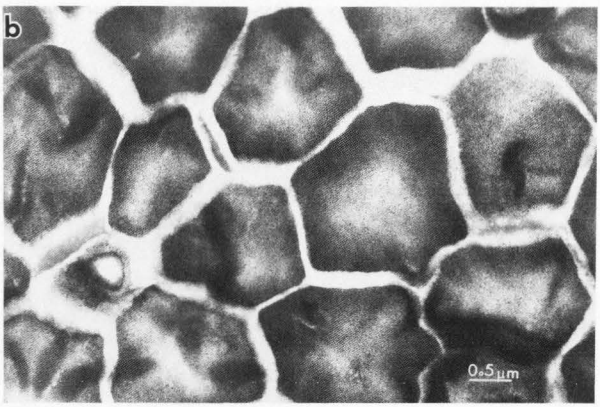


Figure 6. Freeze fracture replica (a) and thin sections of sequentially fixed seeds (b) show spherulitic structure of starch. SI = starch interior; Su = starch surface.

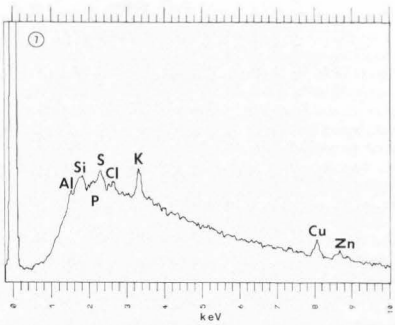


Figure 7. Qualitative X-ray energy dispersive analysis spectrum of the perisperm. Cu and Zn peaks are from instrument parts. Vertical scale is 2 k.

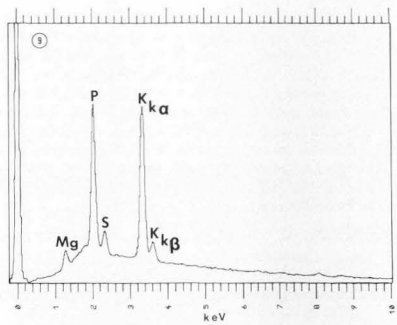


Figure 9. Qualitative X-ray energy dispersive analysis spectrum of the embryo, showing high content of P and K. Vertical scale is 8 k.

The compact spherulitic texture of the starch granules was revealed in both freeze fracture replicas (Fig. 6a) and thin sections of samples that were stained sequentially with glutaraldehyde and then OsO_4 (Fig. 6b). The reasons why quinoa starch granules strongly react with OsO_4 during sequential fixation and not during a simultaneous fixation procedures are unknown. Unlike starch granules of many cereal grains (Buttrose, 1960; Gallant et al., 1972), no concentric rings were observed in quinoa granules.

Matrix protein surrounds the starch granules and interconnects them within the cells (Figs. 5c and d). In most TEM preparations, the starch granules pulled away from the surrounding protein which may suggest weak bonding between those two components. No obvious protein bodies were observed. Perisperm cell walls appear about 1 μm or less in thickness in Figure 5c but less than 0.5 μm thick in Figure 5 d.

X-ray energy dispersive analysis of the perisperm did not reveal high concentrations of any one element (Fig. 7). Low levels of K, S, Cl, and Si predominated.

Embryo

The embryo surrounds the quinoa perisperm (Fig. 1b). The mature embryo is a dicotyledon. Transmission electron micrographs (Fig. 8a and b) of the cotyledonary cells show a complex structure consisting of lipid bodies, protein bodies, nucleus and other organelles necessary to carry out the degradative and synthetic functions involved in the transformation of the seed into a plant. The protein matrix of some protein bodies is granular suggesting that water imbibition during fixation may have initiated cytoplasmic changes normally associated with incipient germination. It is impossible to chemically fix dry seeds without cell hydration during fixation. The protein bodies usually contain two or more electron transparent globoid inclusions which, in turn, contain electron dense globoid crystals or voids caused by the loss of globoid crystals during thin-sectioning. Lott and Buttrose (1977) have shown that globoid crystals of many seeds are rich in phytin (a salt of myoinositol hexaphosphate).

The major inorganic elements detected in the embryo by X-ray microanalysis were Mg, P, S and K (Fig. 9). High cotyledon phosphorus content is consistent with the hypothesis that phytin is a major component of protein bodies.

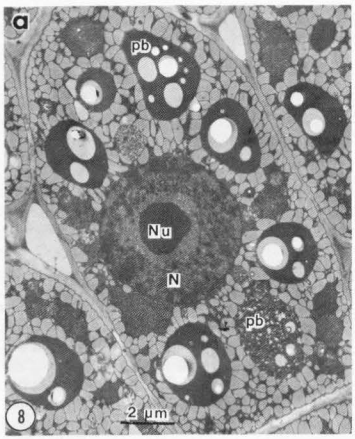
Numerous lipid bodies surround the protein bodies and line the cell periphery. These irregular shaped structures are 0.5 μm or less in their longest diameter.

Effects of Germination

During germination, the embryo unwinds and the cotyledons separate (Fig. 10a). Since quinoa is essentially nondormant (Simmonds, 1965), germination was complete within 24 hrs. The structure of the perisperm did not change dramatically after germination. The starch granules were more loosely packed within the cells than in the perisperm of ungerminated fruit, and the matrix protein was retracted from the starch granules (Fig. 10b). No evidence of significant erosion of starch granule surfaces due to amylolysis was observed by SEM. Some large granules (1 μm or greater) did, however, show evidence of amylotytic degradation at the hilum (Fig. 10c) and the periphery of the granules in transmission electron micrographs (Fig. 10d). Surface digestion holes were 40 nm to 120 nm in diameter (Fig. 10d). Preferential digestion at the hilum suggests that this area is less crystalline than the starch granule periphery.

Figure 8. Embryo of ungerminated quinoa. Transmission electron micrographs in (a) and (b) show protein bodies (pb), globoid inclusions (G), lipid bodies (L), nucleus (N) and nucleolus (Nu).

Figure 10. Germinated quinoa. After 24 hrs. the embryo of the seed unwinds and the cotyledons separate (a). Perisperm cells show retraction of protein from the starch granules (b) and amylolytic degradation (arrows) at the hilum (c) and periphery (d) of the granules.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pb
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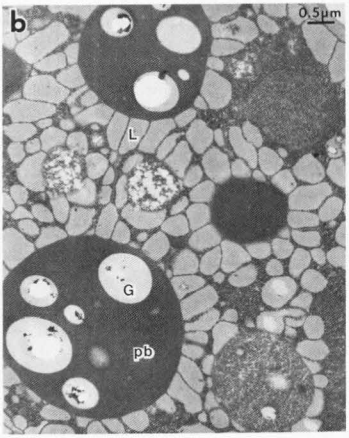
2 µm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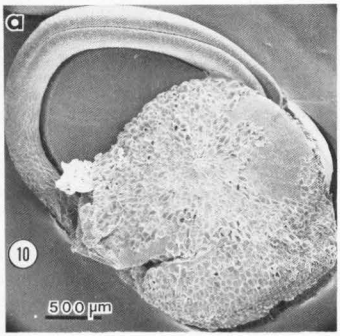
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10
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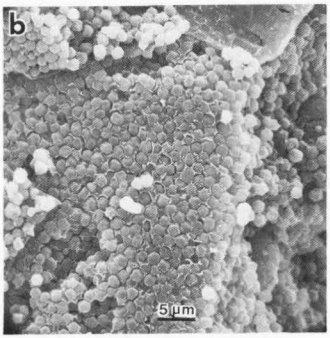
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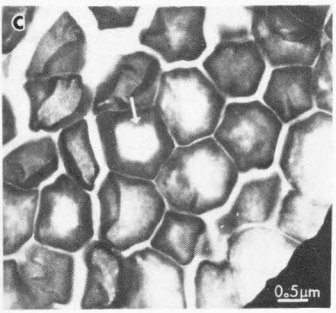
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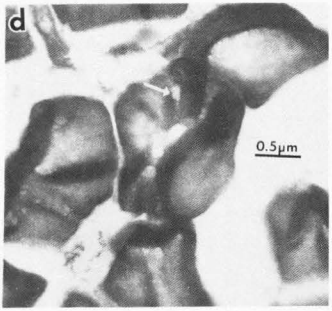
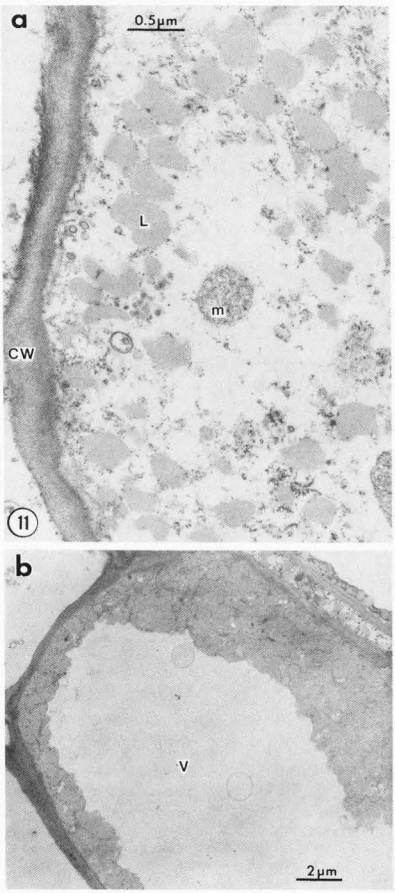

d
0,5µm

Quinoa embryo cells (Fig. 11) showed structural changes typically observed in germinated seeds (e.g. Swift and O'Brien 1972). After 24 hr. germination, most of the protein bodies were hydrolyzed and the remaining organelles and cytoplasm stained more faintly than in the ungerminated embryo cells (Fig. 11a). Cell organelles are surrounded by ribosomes, and many of the cells contain large central vacuoles resulting from the hydrolysis of lipid and protein bodies (Fig. 11b). More lipid bodies remain in the cytoplasm than protein bodies which suggests that during germination utilization of protein is more rapid than lipid.



Summary and Conclusions

The fruit of *Chenopodium quinoa* is consumed in some areas of South America similarly to our consumption of cereal grains in the United States. In fact, all of the literature refers to quinoa as a grain. However, quinoa fruit has some unique chemical constituents and anatomical characteristics that clearly differentiate it from cereal grains.

Quinoa contains saponins which are bitter and possibly toxic. Unlike tannins in sorghum, quinoa saponins are readily removed by washing in water and lightly abrading the fruit. Although the saponins are known to be present in the covering layers of the fruit (Simmonds, 1965), it is unknown whether they exist primarily in the perianth or the pericarp. Such information would be useful if large scale production and processing of this food were considered.

Like cereal grains, starch is the major constituent of quinoa fruit. This storage carbohydrate is located in the perisperm rather than the endosperm. The small polygonal granules in the cells form compound structures with well-defined oblong shapes. Only two articles have been published on the physiochemical characteristics of quinoa starch (Wolf et al., 1950; Atwell et al., 1983). The authors report different gel characteristics and amylose contents for quinoa starch. Discrepancies may be a reflection of varietal differences. Further studies are warranted.

Quinoa fruit is essentially nondormant (Simmonds, 1965). As it imbibes water, germination rapidly ensues, and a dramatic reduction in embryo subcellular organization occurs within 24 hr. Unlike many cereal grains, amylolytic action towards quinoa starch granules is not extensive during germination. Their polygonal structure, small size and aggregation may deter enzyme hydrolysis. Data on cereal starches suggest that some of these factors affect rates of amylolytic degradation (Sandstedt, 1955, Lineback and Pompipom, 1978; Beleia and Varriano-Marston, 1981). Additional knowledge concerning the susceptibility of quinoa starch to amylase action would be helpful in understanding the role of these enzymes in germination as well as providing information of nutritional significance.

Acknowledgements

The authors thank D.A. Fellers, Western Regional Research Center, Berkeley, California, for the quinoa samples.

References

Aguilar RH, Guevara L, Alvarez JO. (1979). A new procedure for the quantitative determination of saponins and its application to several types of Peruvian quinoa. Acta Cient. Venez. **30**, 167–171.

Figure 11. Embryo cells after germinating quinoa for 24 hr. Some lipid bodies (L) still remain but many are partially hydrolyzed (a). Free ribosomes and mitochondria (m) are present; intact protein bodies are rarely seen. Large central vacuoles (v) are present in many cells (b). CW – cell wall.

Atwell WA, Patrick BM, Johnson LA, Gloss RW. (1983). Characterization of quinoa starch. Cereal Chem. **60**, 9–11.

Beleia A, Varriano-Marston E. (1981). Pearl millet amylases. II. Activity toward intact and heated starch granules. Cereal Chem. **58**, 437–440.

Buttrose MS. (1960). Submicroscopic development and structure of starch granules in cereal endosperms. J. Ultrastructure Res. **4**, 231–257.

Clavijo FF, Davila JR, Llepen EC. (1973). Chemical and physiochemical analysis of the quinoa (*Chenopodiaceas*). Bol. Soc. Quim. Peru **39**, 211–216.

Etchevers GG. (1980). Chemical composition of some legumes and *Chenopodiaceas* grown in the Provincia of Nuble. Cienc. Invest. Agrar. **7**, 191–196.

Franke WW, Krien S, Brown Jr. RM. (1969). Simultaneous glutaraldehyde-osmium tetroxide fixation with postosmication. Histochemie **19**, 162–164.

Gallant D, Mercier C, Guilbot A. (1972). Electron microscopy of starch granules modified by bacterial α-amylase. Cereal Chem. **49**, 354–365.

Harborne JB, Simmonds NW. (1964). The natural distribution of the phenolic aglycones. In: Biochemistry of Phenolic Compounds, Academic Press, London, 77–127.

Lineback DR, Pompipom S. (1978). Effects of germination of wheat, oats, and pearl millet on alpha-amylase activity and starch degradation. Die Starke **29**, 52–55.

Lott JNA, Buttrose MS. (1977). Globoids in protein bodies of legume seed cotyledons. Aust. J. Plant Physiol. **5**, 89–111.

Mascorro JA, Ladd MW, Yates RD. (1976). Rapid infiltration of biological tissues utilizing n-hexenyl succinic anhydride (H × SA)/vinyl cyclohexene dioxide (VCD), an ultra-low viscosity embedding medium. 34th Ann. Proc. Elect. Microsc. Soc. Amer., Claitor's Publ. Div., Baton Rouge, 346–347.

Quiros-Perez F, Elvehjem CA. (1957). Nutritive value of quinoa proteins. J. Agr. Food Chem. **5**, 538–541.

Reynolds ES. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212.

Sanchez-Marroquin A. (1983). Two forgotten crops of agroindustrial importance: amaranth and quinoa. Arch. Latinoam. Nutr. **33**, 11–32.

Sandstedt RM. (1955). Photomicrographic studies of wheat starch. III. Enzymatic digestion and granule structure. Cereal Chem. **32**, 17–35 (Supplement).

Seidemann, A. (1966). "Starke Atlas." Paul Parey, Berlin.

Simmonds NW. (1965). The grain Chenopods of the tropical American highlands. Econ. Botany **19**, 223–235.

Swift JG, O'Brien TP. (1972). The fine structure of the wheat scutellum during germination. Aust. J. Biol. Sci. **25**, 469–486.

White PL, Alvistur E, Dias C, Vinas E, White HS and Collazos C. (1955). Nutrient content and protein quality of Quinoa and Canluha, edible seed products of the Andes mountains. J. Agr. Food Chem. **3**, 531–534.

Wolf MJ, MacMasters MM, Rist CE. (1950). Some characteristics of the starches of three South American seeds used for food. Cereal Chem. **27**, 219–222.

Discussion with Reviewers

C.F. Earp: What is the approximate proportions of dry weight of each of the major parts of quinoa?
Authors: No data are available and since the fruits are very small, special dissecting techniques would be necessary to obtain meaningful data on the relative composition of the component parts.

C.F. Earp: What is the difference between a perisperm and an endosperm?
Authors: The endosperm is formed within the embryo sac. The perisperm is derived from the nucellus.

D.B. Bechtel: Since the perianth is so thin, delicate and apparently non-continuous, how can you be sure that the X-ray pattern observed in Fig. 4a is truly the perianth rather than the pericarp "showing" through? How were you able to eliminate the irregular surface effects on the X-ray pattern?
Authors: A spot X-ray analysis was done on the perianth which involves analyzing areas the size of the electron beam. Since the perianth is structurally distinct from the pericarp, it was easy to differentiate the two structures. The perianth is about 2 microns thick. It is quite unlikely that X-ray photons generated at depths greater than 2 microns would be detected. Surface roughness does contribute to variability in X-ray photon collection. This is implicit in all spectra that are generated from unpolished surfaces which is why we call it qualitative X-ray analysis.

D.J. Gallant: What kind of breads are made from quinoa?
Authors: No references gave details of the bread preparations.