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MICROSTRUCTURE OF *NUÑAS*: ANDEAN POPPING BEANS (*PHASEOLUS VULGARIS* L.)

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Abstract

Nuñas, popping beans (*Phaseolus vulgaris* L.), burst and expand when heated rapidly. Differences in seed microstructure between popping and conventional (non-popping) bean genotypes conceivably contribute to popping in *nuñas*. However, the microstructural characteristics which contribute to the popping attribute and sites of expansion have not been identified. Seeds and excised cotyledons of unpopped and popped *nuñas* were examined using scanning electron microscopy (SEM). Protoplasts of unpopped *nuñas* were similar to protoplasts of conventional beans. Intercellular spaces of unpopped *nuñas* were occluded by schizogenous cell walls. The occluded form of intercellular spaces differed distinctively from the open form in popped *nuñas* and untreated conventional beans. The expansion of cotyledon mesophyll in popped *nuñas* came primarily from expansion of cell walls and secondarily by expansion of the intercellular spaces. Cell wall thickness and dimensions of protoplasts were not changed during popping. Expansion of cell walls away from protoplasts created intracellular voids. SEM images indicated that starch granules (grains) in popped *nuñas* were generally not altered by popping. Starch granules did not gelatinize or melt during popping as indicated by retention of birefringence. In contrast to popcorn (*Zea mays* L.), starch granules did not contribute to expansion of popped *nuña* cotyledons.

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Introduction

Nuñas are a type of common bean (*Phaseolus vulgaris* L.) which burst and expand during rapid heating (Hyland, 1968; Zimmerer, 1985; National Research Council, 1989). Popping beans have been and still are grown and consumed almost exclusively at high elevations in the Andes of Peru and Bolivia. In traditional Andean cooking, *nuñas* are roasted in sand or oil (Zimmerer, 1985,1987). *Nuñas* can also be popped in hot air or microwave ovens (National Research Council, 1989; van Beem and Spaeth, 1989). The advantage of *nuñas* over conventional (non-popping) bean genotypes is a shorter cooking time. This difference is especially important at fuel-scarce high elevations. Popping of *nuñas* requires less than 5 min, while cooking of beans in boiling water may require more than an hour for cotyledons to become soft. *Nuñas* do not require additional cooking after popping.

While *nuñas* are an ancient crop (Zimmerer, 1987; National Research Council, 1989), they are relatively unknown outside of the Andean Region and have received little scientific study. The mechanism of popping and sites which contribute to volumetric expansion during popping of *nuñas* are unknown. In contrast, the mechanism and sites which contribute to expansion of popcorn have been studied (Hoseney et al., 1983). The expansion of popped tissue relative to unpopped tissue is substantially greater for popcorn (Hoseney et al. 1983) than the doubling of volume observed for *nuñas* (van Beem and Spaeth, 1989). In popcorn, the starch granules (grains) expand and contribute substantially to bulk expansion of the popped tissue (Hoseney et al., 1983). Large numbers of small fractures in popped corn endosperm also contribute to volumetric expansion (Hoseney et al., 1983). While the microstructure of conventional common bean cultivars has been studied (Varriano-Marston and Jackson, 1981; Swanson et al., 1985; Hughes and Swanson, 1985; Spaeth, 1987a,1989), no information on the microstructure of *nuñas* was found in the literature.

Characteristics which allow *nuñas* to pop are also unknown. Zimmerer (1985) suggested that differences in density between *nuñas* and conventional beans might be partially responsible for popping. A National Research Council report (1989) derived from an informal survey of researchers familiar with *nuñas* indicated that the quality or quantity of starch in *nuñas* may differ from conventional non-popping beans and be responsible for expansion of *nuñas* during popping.

The mechanism of popping is of interest for analysis of popping and because characteristics which permit popping may also influence physiological processes in seeds during maturation, storage, imbibition and germination. Scanning electron microscopy (SEM) is an excellent tool for examining

structural characteristics which may contribute to popping of *nuñas* and sites of expansion.

The objective of this research was to observe tissue and cellular structure of *nuña* seeds before and after popping. *Nuña* microstructure was compared with published descriptions of popcorn microstructure (Hoseney et al., 1983) to identify features which may allow *nuñas* to burst and expand. SEM was used to examine starch granules, protein bodies, protoplasm, cell walls and intercellular spaces of unpopped and popped cotyledons of Nuña Pava and unpopped cotyledons of five additional *nuña* cultivars and three conventional non-popping bean cultivars.

Materials and Methods

Sources of the beans (*Phaseolus vulgaris* L.) examined in this study are given in Table 1. *Nuñas* were field-grown in the highlands of Peru where cultural practices and environmental conditions are conducive to production of *nuñas* which pop well. *Nuñas* were held at ambient temperature and relative humidity before popping and preparation for SEM. *Nuñas* and conventional beans had water contents of 0.05 to 0.06 g H_2O/g seed (dry wt. basis) before popping. Seeds were dried in an oven for 24 h at 100°C for determination of water content.

Individual beans were popped by heating for 2.5 to 4 min at full power in a 1600 watt, 2450 MHz Kenmore microwave oven (Sears Roebuck and Co.).[3] Beans were observed visually during heating and removed from the oven after complete expansion. Microwave preparation of beans for SEM avoided contamination with oil (Hoseney et al., 1983). Microwave preparation also minimized artifacts caused by agitation or redistribution of material by air currents during hot-air popping.

Beans studied with SEM were mature air-dry or popped oven-dry tissues, therefore, fixation, freeze fracture, and critical-point drying were not required (Spaeth, 1987a). Cotyledons were fractured by hand using single-edge razor blades. Beans were sputter coated with gold and observed and photographed at 20 kV on an Hitachi S-570 Scanning Electron Microscope.

Birefringence of starch granules was examined using a Zeiss Photomicroscope II.

Cotyledons from six Nuña Pava seeds and two seeds each of the five remaining *nuña* and three conventional cultivars were examined.

Results

The cotyledons of *nuñas* heated in a microwave oven not only burst the testa and expanded, but also cracked so that the shape of beans was altered as a result of the heating treatment (Fig. 1). The expansion of microwave popped mesophyll excluding the large cracks was similar to that observed for *nuñas* heated in oil or hot air (van Beem and Spaeth, 1989).

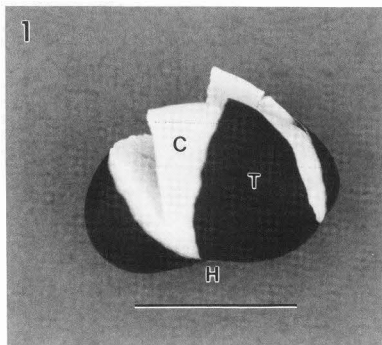The cracks in microwave popped cotyledons ranged

Fig. 1. Popped *nuña* with cracked cotyledons (C), testa (T) and hilum (H). Bar = 10 mm.

Table 1. Sources of beans.

| Cultivar Name	Country, Province	Biological Status
Nuña Pava	Peru, Cajamarca	Landrace
Nuña Parcoyana	Peru, Cajamarca	Landrace
Nuña Huevo de Huanchaco	Peru, Cajamarca	Landrace
Nuña Mani Roja	Peru, Cajamarca	Landrace
Nuña Cuzco Cafe	Peru, Cuzco	Landrace
Nuña Cuzco Blanco	Peru, Cuzco	Landrace
Calima	Colombia, Valle	Bred line
Liborino Redondo	Colombia, Nariño	Landrace
Bolon Blanco	Colombia, Nariño	Landrace

from large, easily visible cracks to small checks. The large cracks were generally transverse with respect to the long axis of the bean (Fig. 1). The epidermis near the large cracks exhibited many small checks roughly parallel to the larger cracks (Fig. 2a). Outside this region, the epidermis was not checked. The mesophyll tissue fractured through cell walls rather than separating between cells at the middle lamella (Fig. 2b). Protoplasts adhered to the fracture cell walls or were pulled away and left empty cell walls. Fractures also pulled vascular bundles apart.

Cotyledon tissue of unpopped *nuñas* fractured through protoplasts of mesophyll cells (Fig. 3a). Cell walls surrounded protoplasts that contained starch granules, protein bodies

Fig. 2. Nuña Pava cotyledon which fractured during heating in the microwave oven. Low magnification (2a) with larger cracks (C) extending into cotyledon mesophyll, the epidermal surface (E), and surface checks (SC) in the epidermis. Bar = 1 mm. Detail of large crack (2b) with protoplasts (P), empty cell walls (W) and a fractured vascular bundle (VB). Bar = 100 µm.

Fig. 3. Fracture surfaces of Nuña Pava cotyledons (fractured for SEM, not a result of popping). Unpopped (3a) with cell walls (W), interior of contact cell wall (ICW), starch granules (SG), large concavities (LC), protein bodies (arrow), and intercellular spaces (IS). Popped (3b) with exterior of protoplast (EP), contact cell wall (CW), schizogenous cell wall (SW), voids (V) between the protoplast and cell walls, intercellular spaces (IS), and projections (Pj) from the surfaces of protoplasts. Bar = 50 µm.

Fig. 4. Transverse fractures through intercellular spaces (IS) of *nuña* cotyledons. Unpopped Nuña Parcoyana (4a) with exterior of protoplast (EP), schizogenous cell wall (SW), contact cell wall (CW) and vertices of the intercellular spaces (arrows). Popped Nuña Pava (4b) with interior surfaces of empty cell walls (ICW) and circular depressions (arrows) in contact cell walls. Bar = 10 µm.
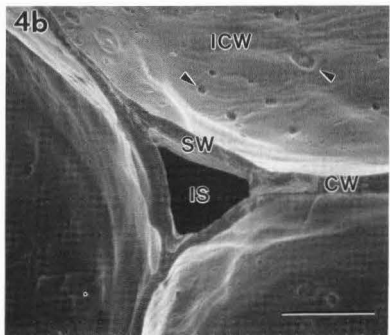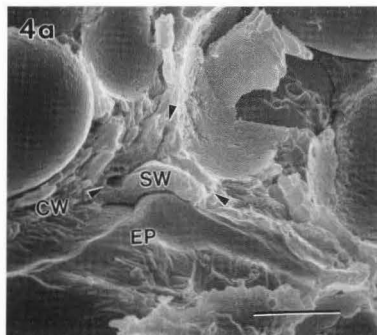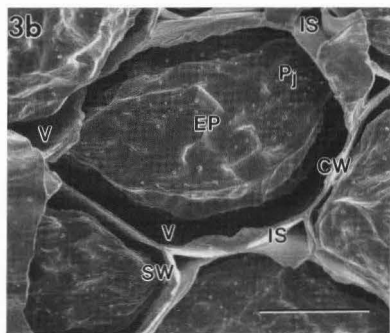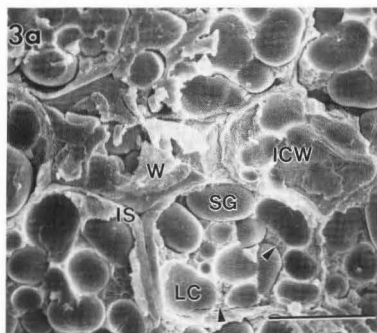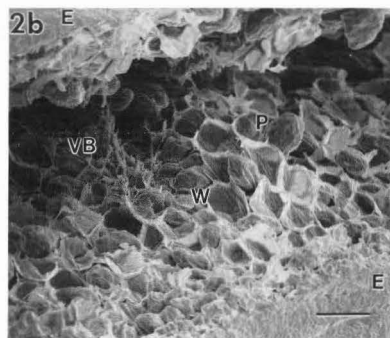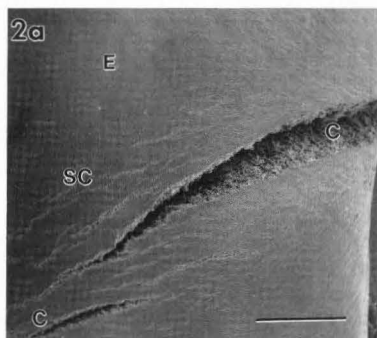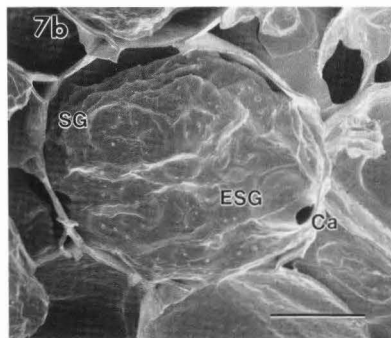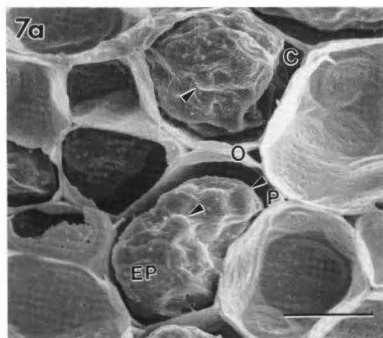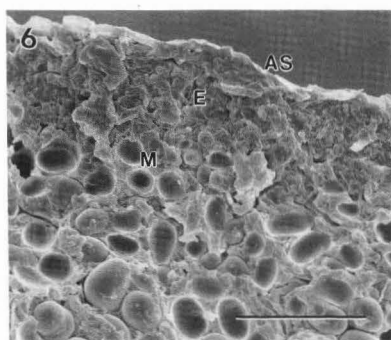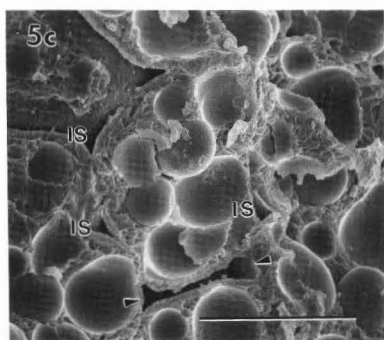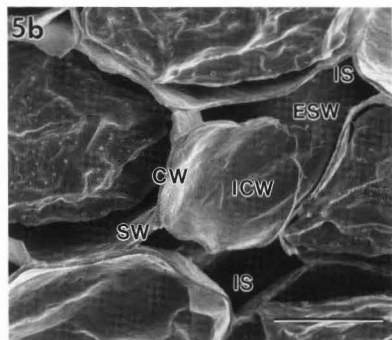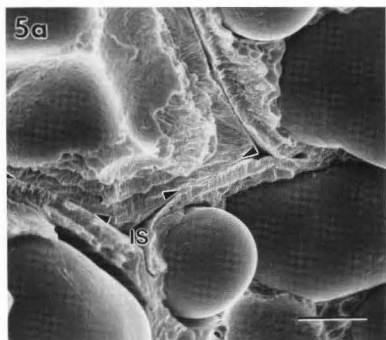
and protoplasm. Cotyledons of unpopped *nuñas* exhibited only a small volume of intracellular voids. Large concave depressions in the fracture surfaces of protoplasts (Fig. 3a) were left by starch granules in the complementary fracture surface. Intercellular spaces were small.

Cotyledon tissue of popped *nuñas* was fractured through cell walls but was not fractured through protoplasts (Fig. 3b). During popping, cell walls expanded, separated from protoplasts, and created intracellular voids. The thicknesses of cell walls were not changed. The separation of walls from protoplasts exposed the exterior surfaces of protoplasts and interior surfaces of cell walls. Popped tissue contained many more voids than unpopped tissue. The largest voids opened between the surfaces of protoplasts and the interiors of cell walls. The distances between the exterior surfaces of protoplasts and interior surfaces of cell walls differed within and among cells but the maximum separation ranged from 10 to 20 µm.

The second contribution to expansion in popped tissue came from the opening of intercellular spaces (Fig. 3b). Schizogenous cell walls occluded intercellular spaces in unpopped *nuñas* (Fig. 4a). Schizogenous walls are the parts of cell walls which separate during seed growth and development, and surround intercellular spaces in mature seeds (Kollöffel and Linssen, 1984, Spaeth, 1989). In contrast, schizogenous cell walls did not occlude intercellular spaces in popped *nuñas* (Fig. 4b). Longitudinal sections through intercellular spaces of unpopped *nuñas* show that the spaces were occluded along much of the length of the spaces (Fig. 5a). Bent or folded segments of schizogenous wall filled the lumens of intercellular spaces. Longitudinal sections through intercellular spaces of popped *nuñas* show that the spaces were open from end to end (Fig. 5b).

The open, continuous network of intercellular spaces in popped *nuñas* (Fig. 5b) was also observed in untreated conventional cotyledons (Fig. 5c). Intercellular spaces in unpopped cotyledons of the additional five *nuña* cultivars exhibited the occluded form of intercellular spaces while the three conventional (non-popping) cultivars exhibited the open form as observed in popped *nuñas*. The epidermal layer of unpopped *nuñas* had fewer voids than the mesophyll tissue of unpopped *nuñas* (Fig. 6).

While occasional intercellular spaces in mesophyll of *nuñas* remained closed or partially closed after popping (Fig. 7a), most intercellular spaces in popped tissue formed an interconnecting network of open spaces. Cracks intermediate in size between macroscopic cracks (Figs. 1,2) and intracellular voids (Figs. 3b,5b,7) were not observed in popped Nuña Pava other than the checks in the epidermis (Fig. 2a).

Protein bodies were not distinguishable from protoplasm in popped *nuñas* (Fig. 7a). Numerous projections were observed on the surfaces of protoplasts in popped Nuña Pava. Depressions in the interior surfaces of contact cell walls (Figs. 4b, 7a) corresponded to the sites where the projections were observed on the surfaces of protoplasts.

Starch granules were not directly visible in popped *nuñas*, because cells of popped beans did not fracture through protoplasts. Starch granules in popped *nuñas* were some-

times evident as bumps beneath the surfaces of protoplasts (Fig. 7a). Size and shape of most starch granules beneath the surface of protoplasts fell within the range of sizes and shapes of starch granules in unpopped *nuñas* (Fig. 7a,b). We observed only one starch grain in the beans studied with SEM that changed markedly during popping (Fig. 7b). The outside diameter of the altered starch granule was larger than the diameters of other starch granules in popped or unpopped *nuñas*. A hole in the surface of the altered starch grain revealed an internal cavity.

The failure of the popping process to alter the external appearance of starch granules prompted an examination of birefringence of starch granules in unpopped and popped *nuñas*. Starch granules in popped *nuñas* exhibited birefringence patterns similar to birefringence patterns of starch granules from unpopped *nuñas* and conventional beans.

Discussion

The volumes of intracellular and extracellular voids in cotyledon mesophyll and epidermis of unpopped *nuñas* were small. The major difference between unpopped *nuñas* (Figs. 3a,4a,5a,6) and conventional beans (Fig. 5c; Spaeth, 1989) was the smaller volume of intercellular spaces in *nuñas* due to occlusion of the spaces by schizogenous cell walls. The relative absence of voids in *nuña* cotyledons was similar to the absence of voids in vitreous endosperm of unpopped corn (Hoseney et al., 1983).

Popping in corn requires steam pressure which builds in the kernel during heating (Hoseney et al., 1983). The occlusion of intercellular spaces in unpopped *nuñas* (Figs. 3a,4a,5a,6) may contribute to popping of *nuñas* by increasing the resistance to flow of steam out of cotyledons, or by absence of space into which steam may expand. Intercellular spaces in popped *nuñas* (Figs. 3b,4b,5b) were more similar to the open, continuous network of intercellular spaces observed in conventional cultivars (Fig. 5c; Hughes and Swanson, 1985; Spaeth, 1987b,1989) than they were to the intercellular spaces of unpopped *nuñas*. The open network of intercellular spaces of popped *nuña* cotyledons may be pathways for escape of steam under pressure. The observation that some intercellular spaces were still occluded after popping (Fig. 7a) indicated that occluded intercellular spaces were present before popping. Cell walls may also constitute barriers to flow of steam during rapid expansion. Resistance to steam flow by occluded intercellular spaces in *nuñas* is a plausible mechanism of popping. Tests of the resistance hypothesis will require *nuñas* and conventional beans produced in one location at one time.

Protoplasts of unpopped *nuñas* (Figs. 3a,6) were similar to protoplasts of conventional non-popping beans (Fig. 5c; Varriano-Marston and Jackson, 1981; Hughes and Swanson, 1985; Spaeth, 1989). The appearance of *nuña* protoplasts was altered by popping as the appearance of conventional bean protoplasts is during imbibition (Varriano-Marston and Jackson, 1981; Hughes and Swanson, 1985; Spaeth, 1989). Projections from the surfaces of protoplasts and depressions in cell walls of popped *nuñas* were similar to

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Fig. 5. Longitudinal fractures through intercellular spaces (IS) in bean cotyledons. Unpopped Nuña Pava (5a) with occluded sections of intercellular spaces (between arrows). Bar = 10 µm. Popped Nuña Pava (5b) with contact cell wall (CW), interior surface of contact wall (ICW), schizogenous cell wall (SW), exterior surface of schizogenous wall (ESW). Bar = 50 µm. Calima (5c), a conventional non-popping cultivar of Andean bean with open section of intercellular space (between arrows). Bar = 50 µm.
Fig. 6. Fracture through unpopped Nuña Pava near the abaxial surface (AS) with mesophyll tissue (M) and epidermal layer (E) lacking air-filled voids. Bar = 50 µm.
Fig. 7. Fractures through popped Nuña Pava cotyledons showing starch granules (arrows) beneath the exterior surface of protoplasts (EP). Figure 7a shows open (O), partially open (P) and closed (C) intercellular spaces. Bar = 50 µm. Figure 7b shows starch grain (SG) and expanded starch grain (ESG) with a cavity (Ca) inside it. Bar = 50 µm.

those observed in beans after imbibition. The sizes of protoplasts did not change during popping (Figs. 3a,b,5a,b).

The expansion of *nuñas* during popping came primarily from changes in the space between cell walls. Cell walls expanded and created intracellular voids around protoplasts (Figs. 3b,5b,7a,b). Schizogenous cell walls of the occluded form in unpopped *nuñas* (Figs. 4a,5a) separated to the open form in popped *nuñas* (Figs. 4b,5b). Intracellular voids contributed more to cotyledon expansion of popped *nuñas* than the open form of intracellular spaces.

The smaller expansion of *nuñas* (van Beem and Spaeth, 1989) relative to the expansion of popcorn (Hoseney et al., 1983) was primarily due to differences in starch granules and the frequency of small cracks. Most starch granules in *nuñas* were not visibly changed during popping of *nuñas* (Figs. 5,6,7) while most of the starch granules in the vitreous endosperm of popcorn expand during popping (Hoseney et al., 1983). The structure of the altered starch granule in Nuña Pava (Fig. 7b) was more similar to starch granules of popped corn (Hoseney et al., 1983) than to other starch granules of either unpopped (Fig. 3a) or popped (Fig. 7) *nuñas*.

The loss of birefringence by starch granules of popcorn indicates that the starch granules have gelatinized during heating (Hoseney et al., 1983). The retention of birefringence in starch granules of popped *nuñas* indicated that the granules did not gelatinize during the popping process. Starch granules in *nuñas*, therefore could not expand in the way starch granules in popcorn expand during popping (Hoseney et al., 1983). Gelatinization and expansion are important determinants of starch digestion rate in the gastro-intestinal tract (Thorne et al., 1983; Würsch et al., 1986). Since starch granules did not gelatinize during popping, starch in popped *nuñas* is probably digested slowly. Reductions in rates of starch digestion are an important consideration for dietary management of diabetes (Thorne et al., 1983).

The reason starch granules did not gelatinize during popping of *nuñas* is not clear. The temperature inside of *nuñas* at time of popping may be lower than the temperature inside of popcorn, or the starch granules in *nuñas* may have a higher temperature for gelatinization than starch granules in the vitreous endosperm of popcorn. Since gelatinization of starch granules depends on temperature and moisture content of the starch, analyses will require measurements of both.

The large cracks in popped *nuñas* were generally transverse with respect to the long axis of the bean (Fig. 2a) similar to transverse cracks formed during imbibition (Spaeth, 1986). Cotyledon mesophyll tissue of popped *nuñas* generally lacked the small cracks (Figs. 2,3,7) which make a substantial contribution to the expansion of popped corn endosperm (Hoseney et al., 1983). The epidermal surface of popped *nuña* cotyledons exhibited small checks (Fig. 2a) which contributed little to expansion.

Nuñas which pop immediately after harvest sometimes fail to pop after a period of aging (National Research Council, 1989). Another aging process of common bean cultivars is the development of the hard-to-cook phenomenon (Varriano-Marston and Jackson, 1981; Swanson, Hughes and Rasmussen, 1985; Hincks and Stanley, 1986). Several mechanisms are reported to contribute to development of hard-to-cook beans including changes in cell wall properties (Hincks and Stanley, 1986) after storage at high temperatures and high humidities. Changes in cell wall properties may also affect the popping process and could explain why popping is sometimes incomplete and variable, even within a *nuña* line.

The occluded intercellular spaces in *nuña* cotyledons constitute an interesting modification of cotyledon tissue from the perspective of seed physiology. The diffusion of gases through dry *nuña* tissue may differ from diffusion of gases through cotyledons of conventional beans because of the differences in pathways for diffusion. The condition of intercellular spaces in *nuñas* before and during dehydration, and during and after rehydration is not known. The occluded intercellular spaces may also alter the hydraulic conductivity and consequently transport of liquid water into cotyledons.

Conclusions

SEM analysis of *nuñas* indicated that the primary sites of expansion were the intracellular spaces between the protoplast and cell wall, and intercellular spaces which changed from an occluded form in unpopped *nuñas* to an open form in popped *nuñas*. The expansion differed from popcorn in that neither starch granules nor small multicellular cracks contributed significantly to the expansion of *nuñas*. The occluded form of intercellular spaces in unpopped *nuñas* differed distinctively from the open form of intercellular spaces in popped *nuñas* and unpopped conventional beans.

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Discussion with Reviewers

W. J. Wolf: If the cell wall expands during popping, wouldn't you expect a narrowing of the cell wall? Have you looked for evidence of changes in the structure of the cell wall as a result of the expansion?

Authors: For a homogeneous, isotropic material, one would expect a decrease in thickness after expansion. Since cell walls are neither homogeneous, nor isotropic, we did not know what to anticipate. We have not observed marked changes in thickness of cell walls. We have not studied changes in cell wall structure as a result of popping in greater detail.

R. W. Yaklich: Do you think the differences you observed in microstructure of popped *nuñas* would be the same at a higher elevation?

Authors: The cooking time required for popping of *nuñas* is reported to increase at high elevations (K. S. Zimmerer, personal communication). Since elevation can influence popping time, elevation may also influence microstructure of popped *nuñas*.

R. C. Hoseney: What is the significance of the fact that popping only takes 4 min to produce a soft bean while cooking in water takes a much longer time?

Authors: Beans become soft during boiling because cells in cotyledons separate from each other along the middle lamella (Hincks and Stanley, 1986). We observed no evidence of cell separation along the middle lamella in popped *nuñas*. Therefore, the process by which *nuñas* become soft during popping differs from the process during boiling. Two different processes of softening apparently develop at substantially different rates.

B. G. Swanson: Is the moisture content of *nuñas* prior to popping similar to unpopped corn?

Authors: The water content of *nuñas* before popping in the current research was below the range of water contents conducive to optimal popping of popcorn (Hoseney et al., 1983). We have not determined the range of initial water contents conducive to popping of *nuñas*.

B. G. Swanson: Is moisture expansion, as steam, responsible for changes in intercellular spaces?

Authors: We hypothesize that steam pressure is responsible for the observed expansion of cells and intercellular spaces, however, tests of this hypothesis need to be conducted.

B. G. Swanson: Is moisture or structure primarily responsible for popping of *nuñas*?

Authors: Moisture and structure both seem to be essential for popping. We suspect that constricted intercellular spaces limit the escape of moisture and cause the *nuñas* to pop after pressure increases in the cotyledons.

B. G. Swanson: Do *nuñas* have a shorter cooking time than conventional beans in water?

Authors: When *nuñas* and conventional beans are both boiled, *nuñas* do not cook more quickly than conventional beans (CIAT, 1989). Softening of *nuñas* during boiling probably takes place by the cell separation mechanism, so the popping mechanism confers no advantage during boiling.

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