Microstructure of Lentil Seeds (Lens Culinaris)

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Abstract

Scanning electron microscopy (SEM) was used to investigate the microstructure of five cultivars of lentil seeds (Lens culinaris). Lentil cotyledons contain spherical starch granules surrounded by protein bodies similar to starch granules and protein bodies observed in cotyledons of other food legumes. Examination of the lentil seed coat in cross-section revealed outer palisade and inner parenchyma layers characteristic of legumes. The subepidermal layer, however, is comprised of hourglass cells and is found primarily in the area surrounding the hilum and the entire lentil seed coat is thinner than the seed coats of most other food legumes. The surface of the lentil seed coat is uneven and covered with distinctive conical papillae. The unique structural characteristics of the lentil seed coat may be partially responsible for the decreased incidence of hardness characteristic of lentils.

Introduction

Lentils are among the oldest cultivated grain legumes and are produced throughout the world. Though lentil production is only of minor importance in global terms, lentils are a very important food crop in certain areas of Asia. Lentils can be divided into two subspecies: macrosperma and microsperma. The macrosperma, found mainly in the Mediterranean region and the New World, are characterized by flat, lens-shaped seeds with yellow cotyledons and pale seed coats which often contain dark brown or black spots, or mottling (Hawtin et al., 1980). Like other legumes, lentils are comparatively high in crude protein (22-36%), supplying approximately twice the protein of cereals, and providing a good complementary lysine-rich protein when consumed with cereals. Lentils are a desirable protein source because they contain few anti-nutritional factors commonly associated with legumes. Low trypsin inhibitor activity (Al-Bakir et al., 1982) and a very low percentage of hard seeds have been observed with lentils, though some flatulence and lectin (hemagglutinin) activity have been reported (Nygaard and Hawtin, 1981). Lentils have the added advantages of rapid hydration, short cooking time and are one of the most easily digested legumes (Nygaard and Hawtin, 1981).

Scanning electron microscopy (SEM) has been used to study legume seed coat surfaces for purposes of seed identification as well as determining the role of the seed coat in water entry. Differences in seed coat pattern have been used to distinguish between members of the sub-family Papilionoideae (Lersten and Gunn, 1982), various lupinus species (Bragg, 1983) and twenty species of the Mimosoideae genera (Baker et al., 1985). In studying selected Papilionoideae, Bridges and Bragg (1983) reported observing that surface patterns varied at different locations on the same seed. Hughes and Swanson (1985) reported that the seed coat surface of common beans evolved and became more complex as the seeds matured.

Wolf and Baker (1972) examined the soybean (Glycine max) seed coat surface and observed numerous pits and pore-like indentations. Wolf et al. (1981) were able to characterize 33 cultivars of soybeans on the basis of seed coat pits and surface deposits. Yaklich et al. (1984) studied permeable and impermeable soybeans, and concluded that the wax/cutin deposit on the seed coat was responsible for impermeability. Sefa-Dedeh and Stanley (1979b)
examined seed coat surfaces of cowpeas and reported observing similar patterns on both the inner and outer surfaces of seed coats.

SEM has also been used to study the hilum, micropyle, and raphe, structures of legume seeds believed to be involved in water entry. Hyde (1954) proposed that the hilum may open and close to regulate internal seed moisture. Kyle and Randall (1963) studied water entry at the hilum, micropyle, and raphe in two cultivars (Great Northern and Red Mexican) of common beans (Phaseolus vulgaris). For Great Northern beans the micropyle was the site of greatest water entry, while in Red Mexican beans the raphe was the most important site. With soybeans, Saio (1976) theorized that a plugged micropyle may be responsible for impermeable soybean seeds, but Yaklich et al. (1984) observed open and closed micropylies in both permeable and impermeable soybean seeds. (Great Northern and Red Mexican) were responsible for impermeable soybean seeds, but proposed that the hilum may open and close to

The most obvious microstructural difference between lentils and other food legumes was observed in examining the seed coat surface. The seed coats of other legumes appear relatively smooth, though generally possessing a characteristic pattern and often being covered with pits and pores or varying amounts of surface deposits. Lentils, in contrast, possess an uneven seed coat surface covered with distinctive conical papillae (Fig. 1). Lersten and Gunn (1982) observed dome-like papillae in Lens culinaris Medikus, quite different from the projecting, conical papillae observed in the five cultivars of lentils investigated here. All five lentil cultivars examined contained papillae structures. In three cultivars (Brewer, Tekoa, and Chief), the papillae were covered with extensive surface deposits (Fig. 2), while two other cultivars (Laird and Red Chief) had relatively few surface deposits (Fig. 1). Though generally scattered, the surface deposits often appeared in sheets which covered all but the top of the papillae (Fig. 3). Surface deposits were present on all seed coats but appeared to be more common on spotted or mottled seeds. The Tekoa cultivar, for example, has a clear seed coat along with extensive surface deposits; in portions of the lentil seed coat the papillae appear mushroom-shaped (Fig. 4). However, careful examination reveals that

Materials and Methods

Lentil seeds (Lens culinaris) examined were provided by the USDA Plant Germplasm Introduction and Testing Laboratory, Pullman, Washington, from seeds grown during the 1983 growing season. All five cultivars studied had pale yellow-green seed coats with two cultivars (Chilean, Brewer) having varying amounts of black spotting or mottling while three cultivars (Laird, Tekoa, Red Chief) had clear seed coats. In order to examine the cotyledon and seed coat in cross-section, the lentils were freeze-fractured. Fractures were initially fixed for 24 h in an aqueous solution of 4% formaldehyde, 1% glutaraldehyde in phosphate buffer (pH 7.0), and dehydrated in a graded ethanol series (30–100%). The lentils were placed in an ethanol-containing pouch in liquid nitrogen and fractured with a razor blade. Fractured seeds were critical point dried in carbon dioxide (Bomar SP-1500), and glued to aluminum stubs. For viewing the exterior of the seed coat, whole lentil seeds were glued to aluminum stubs. All samples were sputter-coated with 300 A gold (Hummer-Technics), viewed and photographed with an ETEC U-1 scanning electron microscope (Hayward, CA) at 20 kV.

Results and Discussion

The most obvious microstructural difference between lentils and other food legumes was observed in examining the seed coat surface. The seed coats of other legumes appear relatively smooth, though generally possessing a characteristic pattern and often being covered with pits and pores or varying amounts of surface deposits. Lentils, in contrast, possess an uneven seed coat surface covered with distinctive conical papillae (Fig. 1). Lersten and Gunn (1982) observed dome-like papillae in Lens culinaris Medikus, quite different from the projecting, conical papillae observed in the five cultivars of lentils investigated here. All five lentil cultivars examined contained papillae structures. In three cultivars (Brewer, Tekoa, and Chief), the papillae were covered with extensive surface deposits (Fig. 2), while two other cultivars (Laird and Red Chief) had relatively few surface deposits (Fig. 1). Though generally scattered, the surface deposits often appeared in sheets which covered all but the top of the papillae (Fig. 3). Surface deposits were present on all seed coats but appeared to be more common on spotted or mottled seeds. The Tekoa cultivar, for example, has a clear seed coat along with extensive surface deposits; in portions of the lentil seed coat the papillae appear mushroom-shaped (Fig. 4). However, careful examination reveals that
Lentil Seed Microstructure

Figs. 1-4. Lentil seed coat surfaces. Fig. 1 shows the papillae covered seed coat free of debris, Fig. 2 shows scattered debris (+), Fig. 3 shows sheet-like debris (→) and Fig. 4 shows debris attached to the tips of the papillae (+). Bar = 5 μm.

Fig. 5. Lentil hilum (H), micropyle (M) and hilar fissure (F). Bar = 200 μm.

the mushroom-shaped papillae are merely conical papillae with disc-shaped debris attached to their tips.

Lentils possess a long, narrow hilum with a micropyle at one end (Fig. 5). The hilar fissure was open on most lentils studied, but in one case was covered with what appeared to be a remnant of the funiculus that had failed to separate. The micropyle of lentils was generally closed or only slightly open (Fig. 6). Examination of the hilum in cross-section revealed characteristic two layers of palisade cells and an unusually thick layer of parenchyma cells causing the hilum to be elevated (Fig. 7). A narrow, elliptical layer of cells known as the tracheid bar runs the length of the hilum under the hilar fissure (Fig. 7). Close examination of the tracheid bar revealed bordered pits similar to the pits observed by Lers ten (1982) without any warts or vestures (Fig. 8).

Cross-sectional examination of the seed coat away from the hilum revealed a discrete outer
palisade layer consisting of a single layer of long (25–30 μm), tightly packed, vertical cells (Fig. 9). A distinctive subepidermal layer of hourglass cells was observed adjacent to but not immediately underneath the hilum (Fig. 10). The hourglass cells are relatively long (30–40 μm) near the hilum, but become progressively shorter away from the hilum and eventually change structural appearance. In portions of the lentil seed coat away from the hilum, gaps or openings were observed immediately beneath the palisade layer (Fig. 9). The seed coat gaps or openings are often difficult to distinguish from the parenchyma layer, but appear to be subepidermal hourglass cells which are shorter, wider and much less distinctive than those observed near the hilum. Though quite thick near the hilum, the lentil seed coat slightly thicker than the most other food legume seed coats (Swanson et al., 1985).

A linea lucida or light line was observed in the palisade layer immediately beneath the seed coat surface of some lentils (Fig. 9). With careful examination at higher magnification, the linea lucida appears not to be structural in nature.

Like other non-oil seed food legumes, lentil cotyledons contain numerous tightly packed storage cells containing large (20–40 μm), spherical starch granules embedded in a protein matrix (Fig. 11). Numerous intercellular spaces surround each of the cotyledon cells. Cell walls can be easily identified, but the middle lamella is not readily distinguishable (Fig. 11).

Conclusions

Lentil seeds are microstructurally similar to the seeds of other food legumes in many ways; however, structural differences are apparent in the seed coat with lentils possessing a papillae-covered seed coat surface, a subepidermal layer that is only clearly visible near the hilum, and a relatively thin seed coat. Additional research is needed to determine if the unique seed coat characteristics of lentils are responsible for lentil's reduced susceptibility to hardening.

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References


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Discussion with Reviewers

R. W. Yaklich: Are the surface deposits derived from the pod endocarp?
K. Saio: Were the structures of the mushroom-shaped papillae caused by contamination of disc-shaped debris? Where does such structural debris come from?
W. J. Wolf: The disc-shaped material on the surface of Tekoa cultivar seed coat is very unusual. Have you examined the interior surface of seed pods of this cultivar? Perhaps more of this material can be found there. Can you rule out microorganisms or fungicide coating given to the seeds by the USDA Plant Germplasm Introduction Testing group?
Authors: The USDA Plant Germplasm Introduction and Testing Laboratory reports that the lentil seeds provided to us were untreated. We were unable to examine pod endocarp because the lentil seeds were supplied without pods. Although microorganisms are a definite possibility, we believe that debris from the endocarp is the most likely source of the surface deposits.

R. W. Yaklich: How does a photograph of an open micropyle differ from a closed micropyle?
Authors: Open and closed micropyles, are not always easily distinguished. For the purposes of this investigation we considered micropyles to be open if there was any visible sign of an opening for water to enter. Closed micropyles, in contrast, were totally closed or fused shut so that no opening was visible.

K. Saio: In Figs. 2 and 3 pit-like structures are observed on the feet of most papillae. Are these artifacts, such as cracking, during specimen preparation or are they natural?
Authors: We believe the pits you are referring to are natural features of the lentil seed coat surface.