

Characterization of Maize Grains (*Zea mays* L.) And Their Living Organisms Entrance Sites By Scanning Electron Microscopy

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Abstract:

Microscopy techniques are used for of grains / seeds characterization, and can also be applied to assess damages caused by microorganisms / insects for a more efficient control methods application. The aim of the work was to obtain information by scanning electron microscopy (SEM) of the structural organization of maize grain (*Zea mays* L.), as well as possible sites of contaminants (living organisms) access to edible parts by SEM. Longitudinal and transversal cuts fixed on stubs and coated with a gold layer were SEM observed. It was registered the dominant vitreous & starch endosperms morpho-histology (hard texture rich in starch granules). Also, the pericarp (external / internal layers), peduncle (the maize to cob link) and the germ structures including the main region of organism's proliferation especially fungi spores and hyphae.

Key Word: Fungi; Maize; Starch; Insects; Characterization

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I. Introduction

Maize is a monocotyledonous, belonging to the family *Poaceae*, Subfamily *Panicoidae*, genus *Zea* and species *Zea mays* L. (Siloto, 2002). It is an herbaceous, monoecious plant, characterized in an annual plant for having its complete cycle of 4 to 5 months (Pons; Bresolin, 1981). These grains are generally yellow or white in color, and may have other colors, from red, purple to black. Its average composition on a dry basis is 72; 9.5; 9 and 4% of starch, proteins, fiber and oil (Paes, 2006).

Maize seed consists of pericarp (fiber layer surrounding the seed), germ (rich in protein and oil) and endosperm, which differ in chemical and organizational composition within the grain (Magalhaes, 2002). The endosperm represents approximately 83% of the dry weight of the grain, organized in the form of granules (Paes, 2006). The pericarp (outer layer) is derived from the wall of the ovary and can be colorless, red, brown or variegated (Magalhaes, 2002).

Based on the distribution of starch granules and protein matrix, the endosperm is classified into two types: floury and vitreous (Paes, 2006; Dernadin; Silva, 2008). These designations refer to the appearance of the endosperm in the grains when subjected to light. In the floury endosperm, the vacant spaces allow the passage of light, giving the material opacity. Conversely, the vitreous represents the absence of spaces between the starch granules and the protein matrix promotes the reflection of light, resulting in a vitreous aspect to the endosperm observed in these conditions (Shotwell; Larkins, 1989; Paes, 2006; Dernadin; Silva, 2009).

This food covers almost all known amino acids, with exceptions to lysine and tryptophan, due to its high composition of carbohydrates (starch) and lipids (oil) it is considered an energetic food for human and animal diets (Imform, 1993; Matos, 2007). Maize oil has in its composition fatty acids, of great importance for the human diet, mainly for the prevention of cardiovascular diseases and the fight against high serum cholesterol (Strecker et al, 1990; Matos, 2007).

Another important aspect of lipids in maize is related to the content of carotenoids and tocopherols (vitamin E). Due to the lignocellulosic content of the green maize grain and in the whole derivatives of the dry grain, these products are considered indispensable sources of fibers, especially of the insoluble type (hemicellulose, cellulose and lignin) (Liebenow, 1986). These characteristics make it recommended in the human diet for cholesterol control, improvement of intestinal constipation, prevention of bowel cancer and diverticulitis, therefore having important functional value (Paes, 2006).

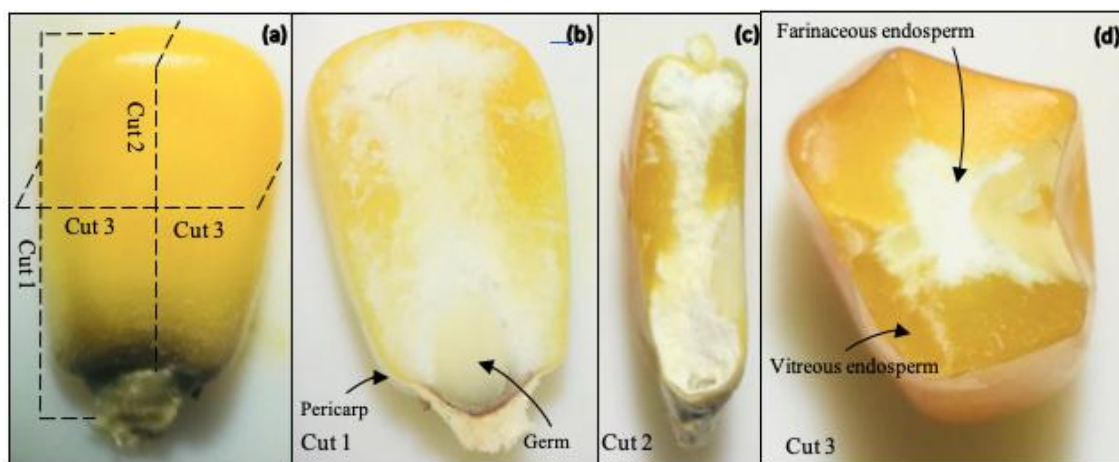
Only a few studies have been carried out for maize grains by scanning electron microscopy (SEM). Considering that the SEM enables knowledge through observation in amplifications of up to 5,000 times, the structure of a food, as well as the perception of the presence of contaminants, invisible to the naked eye and / or light microscope, this work aimed to investigate the structural organization of the maize grain (endosperm and germ) as well as the main sites of possible contaminants (access to edible parts by living organisms) by SEM.

II. Material And Methods

The maize grains (Figure 1) were collected and kindly provided by the Integrated Agricultural Development Company of Santa Catarina - CIDASC, following the Ministry of Agriculture (MAPA) collection protocol (2007).

Its preparation for the microscopic analysis was carried out at the Laboratory of Mycotoxins and Food Contaminants at the Federal University of Santa Catarina. It consisted of the separation of parts of the grains through cuts (a) longitudinal and (b) transverse (Figure 1) following the protocol for processing samples for SEM according to the method of Kluczkovski and Scussel (2015), with subsequent fixation in stubs and coating with layer (40 nm) gold with the aid of vacuum on a planetary basis carried out at the Central Laboratory of Electronic Microscopy (LCME) of UFSC. After coating with gold, the grain was observed in SEM in different amplification.

Figure 1 – Maize grain (*Zea mays L.*): (a) whole and after going through the (b/c) longitudinal, (b) and (c) crosscuts.

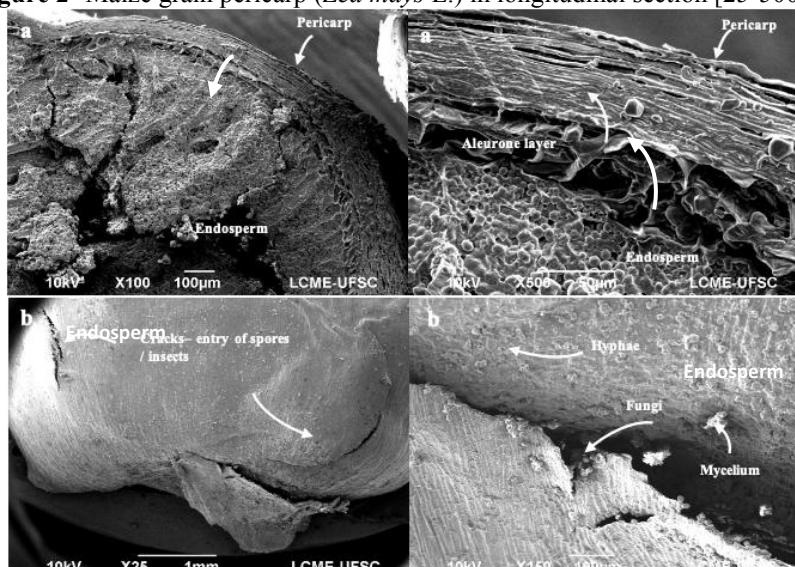


III. Result And Discussion

The maize kernels morpho-histological characteristics are shown in Figures 2 - 5, below (with its pericarp, dominant vitreous & starch endosperms (hard & dusty textures) and germen, including peduncle.

(1) Pericarp (Figure 2): through SEM images, it was possible to observe the pericarp, a structure that protects the other structures of the grain. Through the images we can observe a thick structure, which is formed by the epidermis, mesocarp, crossed and tubular cells (Britannica, 1996). In addition to the pericarp, the aleurone layer, a protein that covers the endosperm, was observed (Germaniet al., 1993).

Figure 2 -Maize grain pericarp (*Zea mays L.*) in longitudinal section [25-500X].



(2) Endosperm: as expected, maize starch showed a combination of granules of varied sizes, predominantly rounded. It was observed that the surface of the granules is shown to be flat, except for some striations in granules, according to the characteristics of the models proposed by Oates (1997) and Sujka&Jamrow (2009), referred to as a “billiard ball hairy”, that is, the surface of the starch granule is not smooth, but with ridges projected outwards.

(a) *Starch (Figure 3):* it can be seen that in this type of endosperm the starch granules are rounded and dispersed, with no protein matrix surrounding these structures, that is, the starch granules are loosely associated with the protein matrix.

(b) *Vitreous (Figure 4):* for this type of endosperm, a dense protein matrix was observed, where the starch granules are densely compacted within the protein matrix, with structured protein bodies, which involve polygonal starch granules, not allowing spaces between these structures.

Figure 3 –SEM micrographs of maize(*Zea mays L.*) starch endosperm: (a) longitudinal and (b) cross sections [40-500 X].

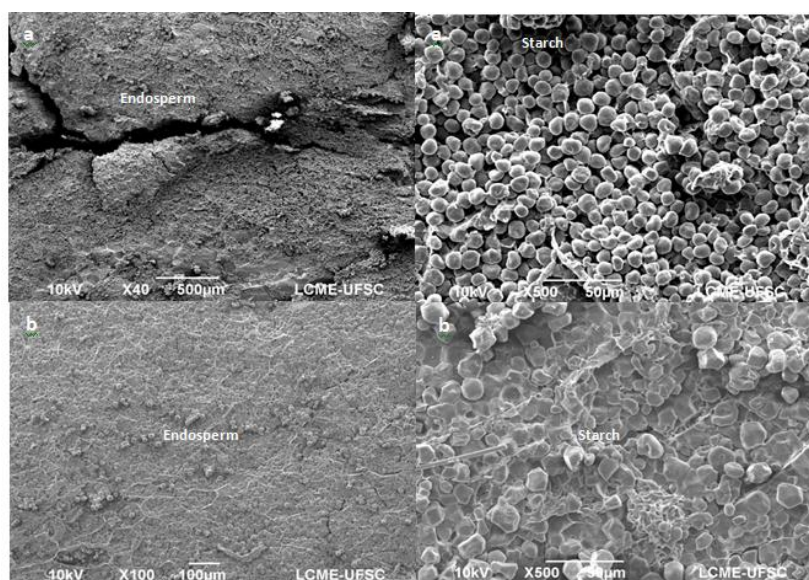
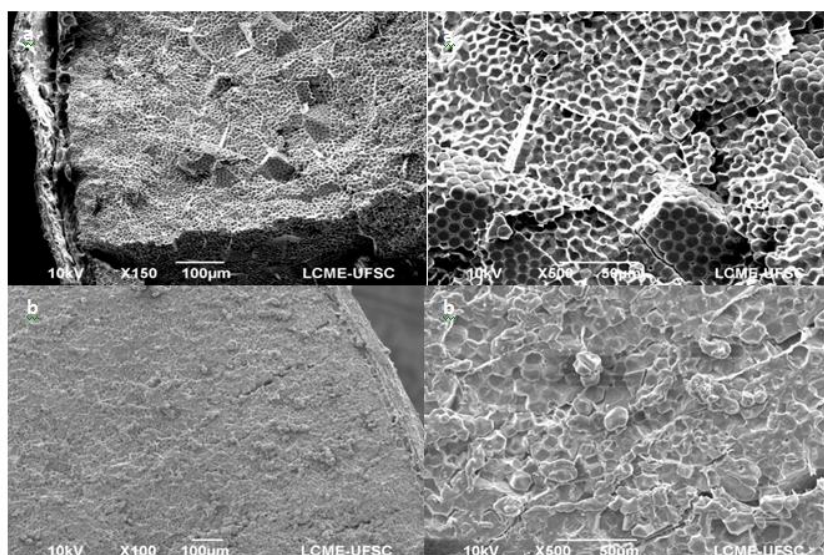


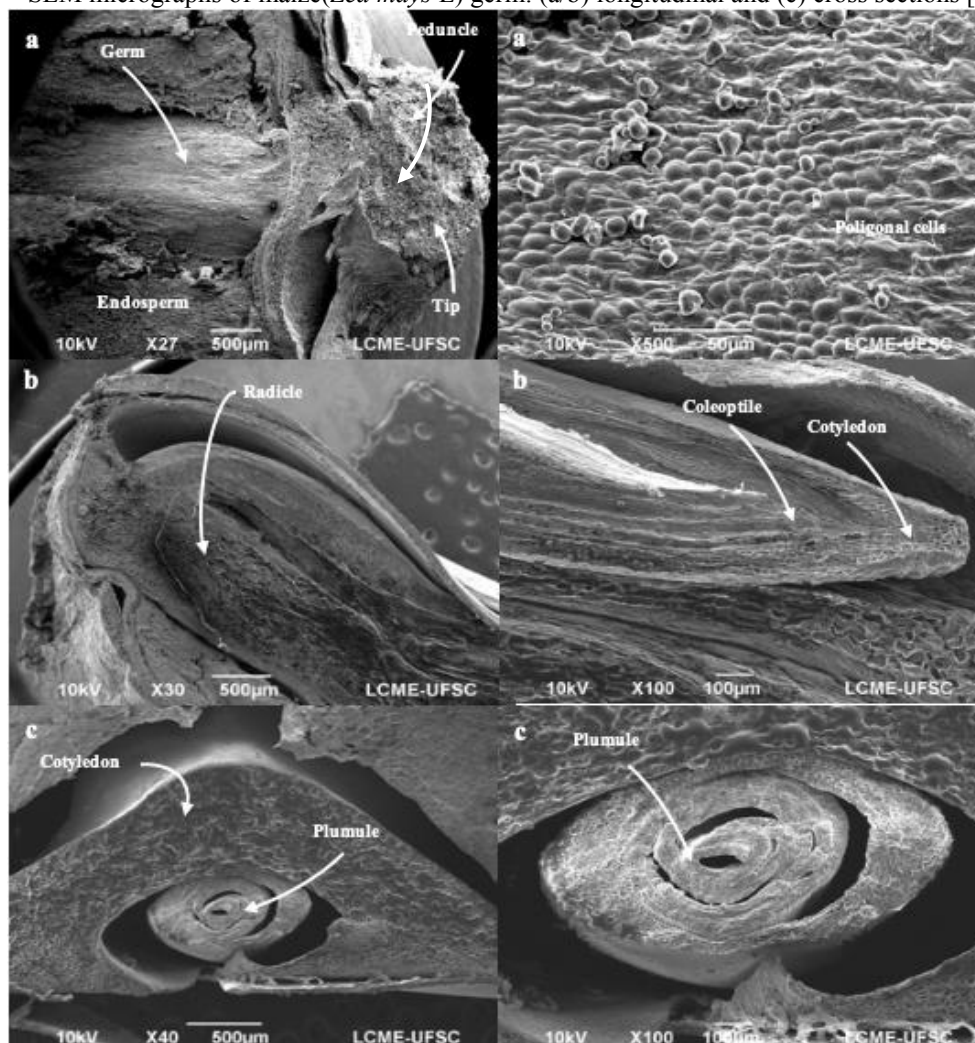
Figure 4 – SEM micrographs of maize(*Zea mays L.*) starch endosperm: (a) longitudinal and (b) cross sections [100-500X].



(3) Germ (Figure 5): the embryo makes up 11% of the total weight of the seed and consists of a plumule (embryonic plant) and the scutellum (cotyledon or seed leaf). Through the cuts and subsequent observation by

SEM it was possible to observe the cotyledon, coleoptile, radicle and plumule of the maize grain. According to Mahanna et al. (2014), the cotyledon is responsible for the first embryonic leaves. The plumule is the set of the apical bud and the primary leaves, and is protected by a sheath, the coleoptile. The radicle is located at the bottom of the germ, and is the primary root of the new plant.

Figure 5 – SEM micrographs of maize (*Zea mays L.*) germ: (a/b) longitudinal and (c) cross sections [27-500X].



Regarding possible contaminants, contamination by fungi was observed. through MEV we are able to observe fungal spores, hyphae and mycelia at the tip of the grain and in cracks, thus providing access to the internal part of the grain by these microorganisms.

IV. Conclusion

The microstructural (pericarp, endosperm and germ) characterization of the internal layers/cell were possible to observe and so the main site for living organism's entrance / infection register – (which leads to deterioration). Therefore, allowing to choose / select prevention methods for more efficient application such as segregation type and modified atmosphere.

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