ADVANCED METHODOLOGY FOR ANALYSIS OF CHANGES IN THE STORAGE LETTUCE SURFACE


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RESUMO – Diversas metodologias são utilizadas para avaliar o comportamento de vegetais, porém ainda existem dúvidas sobre qual a melhor opção para cada finalidade. A microscopia eletrônica de varredura (MEV) tem sido muito utilizada em estudos das características morfológicas de alimentos, principalmente daqueles que possuem curta vida de prateleira e estão constantemente presentes na alimentação dos brasileiros. Com base nestas informações, utilizou-se o microscópio eletrônico de varredura para verificação das alterações ocorridas, em diferentes regiões na superfície de folhas de alface crespa hidropônica, durante 14 dias de armazenamento refrigerado (4 °C), em relação a atividades metabólicas. Nas regiões estudadas foram observados estômatos e monitorado o processo de perda de umidade das amostras (murchamento), em virtude da taxa de respiração e transpiração.

ABSTRACT – Several methods are used to evaluate behavior of vegetables, but there are still doubts about what is the best for each case. The scanning electron microscopy has been used in studies to obtain knowledge about morphological characteristics of foods, especially foods with short shelf life and are constantly present in the diet of Brazilians. Based on this information, the scanning electron microscope was used to check the changes in different regions on the surface of curly lettuce leaves, during 14 days refrigerated storage (4 °C), in relation to metabolic activities. In the studied regions were found stomata and monitoring the humidity loss process of samples (wilting), due to the breathing rate and transpiration.

PALAVRAS-CHAVE: microscopia eletrônica de varredura; alface; taxa respiratória; transpiração.

KEYWORDS: scanning electron microscopy; lettuce; breath rate; transpiration.

1. INTRODUCTION

The feeding of the world's population it is changing in the last years, becoming more targeted to wellness and prevention of diseases. In this context, many leafy vegetables are involved, like lettuce, one of the most consumed, rich in antioxidants like quercetin, kaempferol, luteolin and vitamin C, as also source of fiber that help to regulate the intestinal tract (Llorach et al., 2008).

Lettuce is found minimally processed to facilitate its preparation and consumption, it being offered in washed whole leaves; washed cut leaves; in packaging with modified atmosphere or only in packaging with films (Saracino et al., 1991).

To ensure the safety of food and their shelf life, besides packaging to protection against contamination by microorganisms, insects, rodents, physical stress and control of gas exchange and water steam, also is used refrigeration and high relative humidity (Hardenburg et al., 1990; Saltveit, 1997).
Vegetables shelf life and structural features are directly related to water steam loss rate that occurs by stomata, responsible for transpiration, or by cuticle, a wax layer found on leaf surface to avoid high water losses (Biomania, 2016). Stomata are structures found in lower epidermis of ground plants to control the influence of the high sun incidence, decreasing excessive transpiration rate of vegetables (Biomania, 2016). According to Kader (1992), vegetables present different breath rates, lettuce leaves and cauliflower have breath rate between 20 and 40 mg CO₂/kg.h (5 °C). The minimum processing of vegetables may increase breath rate, causing undesirable changes in color, flavor, texture and nutritional quality of foods (Kader, 1992).

Scanning electron microscopy (SEM) offers differentiated and advanced methodology for analysis the phenomena that occur in the micrometric and submicrometer scale (Kestenbac e Bota Filho, 1994; Maliska, 2005). The use of this equipment allows getting much information from different characteristics of the studied samples, like surface topography, composition and crystallography (Maliska, 2005) and their use is very common in the areas of biology, odontology, pharmacy, engineering, chemistry, metallurgic, physics, medicine and geology (Dedavid et al., 2007).

Thus, this work presents the use of scanning electron microscopy (SEM) for micrographic analysis throughout the shelf life of crispy lettuce samples minimally processed and stored in household refrigerator.

2. MATERIAL AND METHODS

1.1 Feedstock

Crispy lettuces (*Lactuca sativa* L.), grown in hydroponics system in Blumenau/Santa Catarina, were collected in the morning (7 a.m.) and selected based on the characteristics relating to “harvest point to be commercialized”, color pattern, size and absence of defects.

Then, samples were transported in thermal boxes to Food Processing Laboratory, of the Department of Chemical Engineering, FURB, where the study was conducted.

1.2 Methods

**Samples preparation:** After the selection according to size, color and absence of defects, lettuce samples were placed in polyethylene expanded packaging with polyvinyl chloride (PVC) covered and stored in refrigerated incubator (B.O.D.) at 4 °C ± 3 °C and relative humidity of 70% ±10%.

**Scanning electron microscopy (SEM):** To verify changes on the surface of curly lettuce leaves, during 14 days of storage at 4 °C, was used SEM (TESCAN, model VEGA 3 SEM). This methodology use electron beams to explore the sample’s surface, as described by Dedavid et al. (2007). The equipment used was the sample metallizer (QUORUM, model Q150R ES). It was analyzed top region and central part of lettuce leaves surface.

3. RESULTS AND DISCUSSION
The analysis allows to detect large number of stomata on the sample’s surface as seen in Figure 1, concerning to the first day of storage.

Figure 1 – Micrographs of lettuce leaf on the first day of storage at 4 °C; (a) top leaf region and (b) central leaf region, with magnification of 200 times.

The quantity of stomata found may be explained by growth conditions of plants environmental (like the availability of water in the environment) and storage of the minimally processed product (Antunes, 2009; Castro, 2016a). The presence of stomata is related to the environment in which the plant lives, and are positioned in the attempt to reduce the loss of water as steam, but have little taxonomy application (Castro, 2016b).

The study conducted by Takeuchi e Frank (2001), demonstrated that how greater the number of stomata per unit of lettuce’s surface area, greater the chance of entrance of the cells of E. coli O157:H7, influencing microbiological food safety. It may be prevented with good practices during vegetable handling and use of packaging (Gould, 1988).

It was verified low number of stomata on the sixth day of storage as shown in Figure 2. As in the first day, the stomata were open, showing high transpiration (gain or loss of water) which is stomata function in plants, proportional to breath rate (Vieira, 2015). On the sixth day, leaf’s surface had shriveled appearance, according Galvão (2009) this could be caused mainly by physicochemical changes such as the loss of water for the environment.
As shown in Figure 3, on the fourteenth day of storage lettuce leaves were very rough, this may be due to breath activity along the shelf life.

It was observed, in the last storage day, a small number of stomata. According Fonseca (2002) this is natural in vegetables in senescent phase, but that still allowing food’s water transfer to the environment, affecting directly in the vegetables sensory characteristics.

According Galvão (2009) the wilting process, that gives high roughness to the samples, is associated with water loss in vegetables, leading in weight loss and quality. These losses occur due to metabolic reactions that change product texture and the transpiration rate that minimizes the wilting.
process, which can be done with increased of relative humidity in the storage place, decreased of temperature, use of nutrients in the preharvest that influence in plant cell structure and others.

Conti and Tavares (2000) verified that, depending on the growing region, climate and cultivar, there are more or less variation in the number of stomata, the crispy leaf variety has 6585 to 7279 stomata/cm² and butter variety has 4405 to 9487 stomata/cm².

4. CONCLUSION

The methodology used in this study had results that allowed observe and analyze sample’s surface with details of microstructural changes, mainly related to morphological characteristics. Furthermore, SEM stands out when compared with others methodologies, because allows to obtain the estimate of the number of stomata by area and visual structural changes, that are directly related to the development stage of the vegetable, respective breath and transpiration rates.

5. BIBLIOGRAPHIC REFERENCES


