Abstract

Bitter pit is a physiological disorder occurring in apple, pear, and quince whose symptoms are known to develop in storage, appearing several weeks to months after harvest. This disorder causes commercial losses for apple growers as affected fruits are declined or achieve lower prices in the market. Uneven calcium distribution in fruit tissue has been thought to be one of several factors that cause bitter pit. Despite bitter pit has been studied for more than a century, the mechanisms involved in its development are still not well understood. To date, most of the research carried out on bitter pit has been focused on Ca^{2+} deficiency. However, little to no attention has been paid to the expression of other important metabolites indicative of cell status.

Phenolic compounds and proteins are end products of numerous cellular processes occurring in the biological systems as a result of natural defensive reactions against stress and diseases. In the quest to understand bitter pit, the study of these chemical "fingerprints" cannot be avoided, as they can help to better understand the biochemical mechanisms involved in the development of this disorder.

Under this premise, this research has addressed the analysis and comparison of phenolic compounds and proteins present in healthy and bitter pit affected tissues of apple (*Malus domestica* Borkh). For this purpose, several approaches and techniques have been employed.

The analysis of phenolic compounds and antioxidant activity in healthy apples was first carried in order to optimize the methodology (Chapter 1). Even if most of phenolic families in apple could be successfully identified, the methodology employed, high performance liquid chromatography coupled to a UV-DAD detector, presented some limitations to accurately identify all the eluted peaks. As a result, an alternative methodology based on mass spectra detection of target phenolic compounds was employed to compare the phenolic profile of healthy and bitter pit tissues (Chapter 2).

With respect to the study of proteins, the identification of an 18 kDa protein suggested as a potential bitter pit marker by former group studies was first carried out (Chapter 3). The characterization of additional proteins overly expressed in bitter pit tissues was next addressed by means of most sophisticated proteomic-based analytical strategies (Chapters 4 & 5).

Results suggest that down-regulation in the expression of major phenolic compounds and low antioxidant activities were associated with the presence of bitter pit disorder, which may suggest that oxidative activity accompanies bitter pit disorder development. Bitter pit disorder also induced a deep change in the protein profile of affected tissues, expressing a variety of pathogenesis-related (PR) proteins from several families, including Mal d 1 and Mal d 2, two major allergens. Other proteins with diverse cell functions such as tissue desiccation, mitochondrial carrying or protein binding among others were found to be upregulated in bitter pit tissues.