ABSTRACT

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Title of Doctoral Thesis: Evaluation of phenolic substances content in plant material

Scientific community has always been interested in the research of natural substances. The main reasons are a huge diversity of substances themselves and their significant impact on human health. The development of new analytical and extraction methods, including new approaches for sample preparation, enable obtaining more detailed knowledge about the structure and biological properties of plant substances. A significant group consists of substances with antioxidant effects, such as phenolic compounds, which are an important part of human food.

The presented dissertation thesis deals with determination of selected groups of natural substances from the point of view of specific requirements for chromatographic separation, choice of detection technique with regard to the selectivity and sensitivity of the determination, as well as the possibility of applying modern and green extraction approaches.

The first part of the presented work is devoted to the issue of determination of selected plant substances. For this purpose, the results obtained by measuring a set of apple varieties using a diode array detector and a charged aerosol detector were compared. Both detectors enabled quantitative characterization of the monitored substances in the samples. However, comparison of results provided by both methods confirmed significant differences in the sensitivity and also the selectivity of detections, due to a complexity of sample matrix that significantly increased requirements for separation efficiency. A coulometric detection was applied as an additional detection technique which enabled interpretation of sample quality from a functional point of view, based on the content and activity of substances with antioxidant properties.

Another part of the work was devoted to a development of a separation method for determination of structurally similar stevia glycosides using a charged aerosol detector, which enabled sensitive quantification of weakly UV absorbing analytes. For this purpose, several stationary phases based on fully

porous and superficially porous particles, or particles with modification for a separation of polar substances were tested. Since stevia glycosides are small and very polar molecules from a physico-chemical point of view, a separation using hydrophilic interaction chromatography mode was tested in addition to a reversed phase mode. The optimized method was validated for two types of matrices – plant extract and commercial sweeteners. The validated method was used to verify the quality of commercially available stevia sweeteners and to quantify stevia glycosides directly in the plant extract.

The thesis also discusses the possibility of using a modern and green extraction method applying carbon dioxide for extraction of polar phenolic compounds. This method is also compared with the often-applied sonication extraction. The development of both extraction methods was done in several steps also using design of the experiment for detailed optimization of individual extraction parameters. The extraction of a set of dried apple samples by both methods allowed their comparison not only with regard to the overall yield, but also method greenness and practical aspects.

For the needs of a simple, reliable and at the same time complex characteristic of fruit samples, a certified methodology was developed. The methodology describes in a comprehensive way subject of plant analyses on the example of determining phenolic substances in apple samples. The work confirmed importance of applying coulometric detection method and other antioxidant tests, which were mutually complementary and provided important data on the biological value of the samples. The use of diode array detector and a charged aerosol detector was discussed with respect to the quantification of selected analytes. The sensory analysis of the samples was carried out as a part of pomological evaluation.