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**The use and optimization of advanced separation techniques for the extraction and concentration of anthocyanins as an ingredient of functional food**

**Abstract**

Berries, owing to the high content of polyphenols, in particular anthocyanins, possess a beneficial effect on health. Studies have confirmed positive effect of fruit extracts such as chokeberry, elderberry and bilberry on the circulatory system, immune system and anti-inflammatory properties. It has been shown that elderberry fruit extracts as well as purified anthocyanins stimulate the uptake of glucose and fatty acids by skeletal muscle cells, which is helpful in the prevention of type 2 diabetes. Berry fruit extracts, due to their strong antioxidant properties, can slow down the aging process and have anti-cancer properties. Dry extracts from berry fruits are also used in dietary supplements supporting the proper functioning of the eyesight. Extracts with high anthocyanin content are used moreover as the natural food colorant.

The final form of the extracts developed within this PhD thesis is a powder, because the anthocyanins are more stable and the extract has a much higher coloring power compared to the juice concentrate. Almost the entire process takes place at low temperature and the contact with high temperature during spray drying is very short. This results in only slight decrease in anthocyanins amount during the production process.

The research was divided into several stages. In the first stage, efficient conditions for the extraction of anthocyanins from various commercially available raw materials, such as frozen pomace and chokeberry fruits, dried pomace and frozen elderberry fruits, and frozen blueberry

fruits, were developed. It was found that aqueous solutions of citric acid and phosphoric acid are efficient anthocyanins extractant. By replacing the organic solvents, which are commonly used in extraction of anthocyanins, with food grade acids, technological problems at the extract concentration step were avoided. Owing to that, the production costs were also reduced, because in the case of using organic solvents, it is necessary to evaporate these solvents before separation, which is energy-consuming and requires the use of a more complex production facilities.

In the next step, the process of preparative anthocyanin separation on macroporous adsorption resins was investigated. The separation of the extracts from pomace and the fruits of chokeberry, elderberry and from bilberry fruits with different anthocyanin content was investigated under laboratory conditions. The obtained extracts, despite the different contents of anthocyanins in raw materials, were characterized by a high content of anthocyanins in dry matter, in the vast majority exceeding 25%.

In the following stage of the research, the possibility of using pressure-driven membrane techniques for the separation of anthocyanins was investigated. Microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF) ceramic and polymeric membranes were tested. No anthocyanin separation was found on the microfiltration membrane. The use of nanofiltration made it possible to thicken the extract however insufficient increase the anthocyanin content in the dry matter of the extract was found. Attempts to dry the extract after separation with NF failed, which indicated an insufficient reduction in the concentration of mono- and disaccharides which are the compounds with low glass transition temperature. Ultrafiltration was successfully applied. Anthocyanins extracted from selected fruits, despite the molecular weight in the range of 419-744 Da, were efficiently rejected on the UF membrane with the molecular weight cut-off (MWCO) of 1 kDa. The influence of transmembrane pressure, temperature, concentration of the extract and diafiltration on the content of anthocyanins in the dry matter of the extract was investigated. It has been proven that higher transmembrane pressure and lower temperature have a beneficial effect on the retention of anthocyanins on a membrane with a MWCO of 1 kDa. An increase in the content of anthocyanins in the dry matter of the extract was also proven with a higher concentration of the extract and during diafiltration. After separation by ultrafiltration on a 1 kDa membrane, the extracts can be efficiently dried because they do not adhere to the walls of the spray dryer. This proves the reduction of the content of mono- and disaccharides.

The next stage of research work was the implementation of some of the developed methods for production at Greenvit Sp. z o. o. At the present stage, extraction and chromatographic separation have been implemented. Vacuum compaction and spray drying in accordance with the previously used standard procedures made it possible to obtain satisfactory results. In this step, the yields of five batches of elderberry extract and bilberry extract and four batches of chokeberry extract were compared. The total recovery of anthocyanins contained in plant raw materials, except for one batch of elderberry extract, exceeded 75%.

On the basis of the researches, methods of producing chokeberry, elderberry and bilberry extracts were developed in the production scale. Specifications have also been developed for extracts which have already been included in the company's offer

Aronia and elderberry extracts were also subjected to tests on cultured cells. The in vitro anti-inflammatory and antioxidant properties of chokeberry extract on RAW 264.7 macrophages were tested. The cytotoxic properties of two elderberry extracts against tumor cells of the lung (A549), ovary (A2780), breast (MCF-7) and intestine (Caco-2) as well as against non-neoplastic peripheral blood mononuclear cells (PBMC) were tested and compared. Both extracts have been shown to inhibit the proliferation of ovarian, breast and intestinal cancer cells as well as to stimulate the proliferation of PBMCs. However here was no inhibition of lung cancer cell proliferation.

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(miejsowość, data)

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(podpis)