

OPTIMIZATION OF FRUCTANS EXTRACTION FROM *IN VITRO* CHICORY 'HAIRY' ROOTS

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Genetic transformation of plants is stressful factor and can cause a number of changes in biochemical processes in transgenic plants. Increased synthesis and accumulation of reserve compounds, including fructans of different degrees of polymerization (with low molecular weight - LMF, and with high molecular weight - HMF) is one of these changes. Fructans are biologically active compounds which are used in medicine and food industry. Transgenic roots, as well as natural plant material may be the source of LMF and HMF. Biotechnological techniques can increase the content of these compounds. Therefore, the development of effective methods of fructans extraction and fractionation, determination of their content in *in vitro* "hairy" roots, is the actual direction of scientific research. In our experiments we investigated the dependence of fructans extraction efficiency on time, steeping temperature and time of high temperature extraction.

Dried and powdered chicory *Cichorium intybus* L. cv Pala rossa "hairy" roots obtained by *Agrobacterium rhizogenes*-mediated transformation with pCB161 vector were used for research. There were used low- and high-temperature extractions: 1) fructan extraction without heating at +22°C during 0.5, 1 and 24 hours, 2) extraction with heating at +70°C, +80°C and +90°C during 10, 20 and 30 minutes. Fructan fractionation was conducted by two ways: 1) HMF separation by crystallization at +4°C, 2) NMF separation by extraction with 95% ethanol. To determine fructans concentration in the extracts McRary and Slattery method was used.

Based on the experimental data, a mathematical model of fructans extraction process was created. Its adequacy was tested with the Fisher criterion and coefficient of determination. There were optimal parameters of the extraction process chosen using the methods of linear programming. Extraction for 30 minutes at 90°C without steeping is identified as the most technological. It allowed

to extract fructans general amount from transgenic roots ($146 \pm 8,77$ mg/g of root dry weight). Fractioning by refrigeration at $+4^{\circ}\text{C}$ allowed separating the HMF fraction at the amount of 3% of the total fructans, refrigeration using activated carbon to increase the number of crystallization centres allowed to increase the yield of the HMF up to 14% of the total fructans. 18% of the total fructans HMF fraction was isolated as result of fructans two-stage extraction from “hairy” roots (the first stage - extraction by 95% ethanol to separate the LMF, the second stage - the extraction by hot water to separate the HMF).

Optimal mode of fructans obtaining from chicory “hairy” roots is extraction at $+90^{\circ}\text{C}$ for 30 min. Steeping time does not affect any effectiveness for such extraction. The most effective mode of NMF and HMF obtaining from transgenic chicory roots is two-stage extraction with 95% ethanol at $+80^{\circ}\text{C}$ and water at $+90^{\circ}\text{C}$ for the duration of each stage of 30 minutes.