



METHOD OF ISOLATION AND PURIFICATION OF XANTHOPHYLLS

FROM DIATOMS CULTURE

(TECHNOLOGY OFFERS P-256 and P-257)



The subject of the offer is a method of isolation and purification of two xanthophylls: diadinoxanthin (Ddx) and diatoxanthin (Dtx) from diatoms culture. Pigments are obtained with the purity higher than 99%.

Application: HPLC calibration standards, substrates in enzymatic activity essays industrial-scale production of Ddx and Dtx

Xanthophylls

Xanthophylls are yellow, oxygenated carotenoid pigments which occur widely in nature. They are synthesized by plants and microorganisms whereas animals have to obtain them from food. Xanthophylls protect photosynthetic organism against excess light which can lead to formation of reactive oxygen species, damage of photosynthetic apparatus and decrease the efficiency of photosynthesis (so called **photoinhibition**). They have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases. With their antioxidant, anticancer, anti-inflammatory and neuroprotective properties, they found important applications in food and nutrition, cosmetic and environmental industries, as well as pharmacy and medicine. Therefore, they are of great interest to scientists.

How xanthophylls prevent photoinhibition?

The xanthophyll cycle involves the enzymatic removal of an epoxy group from the xanthophyll molecule in high light stress. The product of this reaction has an ability to dissipate the excess of energy from photosynthetic system and hence to protect it from damage. Conversely, under low light conditions, the epoxy group is reattached to the xanthophyll molecule.

Ddx and Dtx are two pigments responsible for the main photoprotection cycle of xanthophylls in diatoms. These compounds represent an interesting target in pursuing an understanding of the photosynthetic process. Unfortunately, the availability of Ddx and Dtx on the market is low and up to date limited as they are sold exclusively in small quantities at extremely high prices to be used as HPLC calibration standards.



Method of isolation and purification of Ddx and Dtx

The solution of the above mentioned problems is JU's 5-step method for the isolation and purification of Ddx and Dtx:

- ✓ culture diatoms in conditions ensuring maximum production of selected xanthophylls,
- ✓ extraction of pigments from diatoms cells,
- ✓ saponification,
- ✓ fractionation of pigments,
- ✓ purification of Ddx and Dtx by open column chromatography.



The main advantages of the proposed method are:

- ✓ high purity level of preparation ($\geq 99\%$),
- ✓ short procedure time – pigments isolation and purification takes about 22 hours,
- ✓ high-efficiency procedure – approx. 50 μg of pigments is retrieved from 2×10^9 diatoms cells,
- ✓ low-cost procedure – approx. price of 1 μg of Ddx is 0,20 € and 1 μg of Dtx is 2,00 € (prices estimated for laboratory scale),
- ✓ simplicity of the procedure – only 5 steps,
- ✓ use of reagents, which are commonly and widely used in laboratory practice,
- ✓ possibility of large-scale culture of diatoms in bioreactors,
- ✓ application of open column chromatography, which is a valued preparative method and only one, which can be used in industrial scale,
- ✓ possibility of the repeated use of chromatographic adsorbent,
- ✓ application of the obtained pigments in all types of analysis, e.g. as calibration standards for HPLC or as substrate in enzymatic activity tests.

Xanthophylls isolation and purification method from diatoms culture is **the subject of a patent application**. The invention is still under development by the scientists from the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. Currently the Centre for Technology Transfer CITTRU is looking for entities interested in licensing and commercial application of the described solution. CITTRU is also looking for the business partners for joint research and development projects involving the above research topic.

For detailed information please contact:

Centre for Technology Transfer CITTRU: Klaudia Polakowska, PhD

Phone: +48 12 6633832, klaudia.polakowska@uj.edu.pl

ul. Czapskich 4
31-110 Krakow/Poland
phone: + 48 12 663 38 30
www.cittru.uj.edu.pl