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Chromatographic Analysis Of Carotenoids And Carotenoid Esters In Tissues Of Aquatic Organisms

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Unique carotenoids are synthesized by each of major phytoplankton divisions. Because the carotenoids are hydrophobic, they bioaccumulate in tissues with high lipid content, and serve as trophic markers for aquatic organisms. Nonetheless, there are analytical challenges to overcome, from background lipids and their metabolites, in determining the carotenoid composition of tissue samples. In most aquatic organisms, triacylglycerides and phospholipids are present at the percent level, whereas pigments are only present at trace, microgram-per-gram levels. Background lipids can interfere with partitioning of carotenoids in liquid chromatography (Minquez-Mosquera, 1992) and can increase the noise level in electrospray ionization mass spectrometry on the positive mode (Breithaupt, 2002). To address this problem, we are developing a solid-phase extraction (SPE) method to separate carotenoids from bulk lipids. Carotenoids from mussel tissues are extracted in acetone; separated from polar lipids using a Toyopearl DEDA-650M ion exchange resin and finally separated from neutral lipids on a Sep-pack silica phase SPE cartridge using a gradient of hexane and acetone. The colored eluate is dried under nitrogen and redissolved in the HPLC solvent. Sample extracts are injected onto a reversed phase C8 Supelco analytical column and analyzed by HPLC-PDA-FL (high performance liquid chromatography-tandem photodiode array-fluorescence detector system). In experiments to date, recoveries of standards from spiked reference samples have ranged from 75 to 110 percent. The overall method is being evaluated with respect to recovery, precision, accuracy, selectivity and analyte stability.