



Analysis of bixin contents in Bixa orellana seeds

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Abstract

Analysis and comparison of immature seeds in this study were particularly important since bixin accumulation is higher in immature than in mature seeds

Materials

HPLC (high pressure liquid chromatographic) Agilent 1100 MWD

Hypersil ODS C-18 reverse phase column (25 cm x 4.6 mm; 5 µm diameter beads).

centrifuge (Sorvall Legend Micro 21R centrifuge. Thermo scientific)

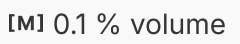
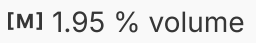
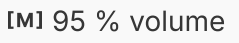
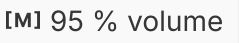
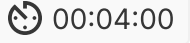
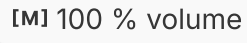
Hypersil ODS C-18 reverse phase column

vortexed

filter using Millex-GV13, (Durapore PVDF, 13 mm diam. 0.22 µm pore size. Millipore, Billerica, MA)

Analysis of bixin contents in *Bixa orellana* seeds

- 1 Quantitative analysis of bixin by high pressure liquid chromatographic (HPLC).

 10 mg of seeds tissue from S1, S2, S3, S4 and S5 were freeze dried powder seeds with  0.8 mL ml of chloroform/water/1:1 (v/v/) and shaking by 1  01:00:00 hr at room temperature, then add  800 μ L chlorform, the tubes were vortexed, centrifuged at 10000 rpm for  00:10:00 and  4 °C (Sorvall Leyend Micro 21R centrifuge. Thermo scientific) and collect bottom phase (non polar extract). Then dry and resuspended in  500 μ L of .  20 μ L of each extract was injected on a Hypersil ODS C-18 reverse phase column (25 cm x 4.6 mm; 5 μ m diameter beads). The mobile phase consisted of solvent A (water containing  0.1 % volume formic acid, pH3) and solvent B (acetonitrile) and the column developed as follows: Step  1.95 % volume solvent A at injection for  00:05:00 ; Step2, linear increase to  95 % volume solvent B in  00:25:00 ; Step 3,  95 % volume solvent A for  00:04:00 ; Step 4, return to  100 % volume solvent B. Samples were detected at 450 nm on a Agilent 1100 MWD detector at a flow rate of  0.8 mL /min at  20 °C . All samples were filtered using Millex-GV13, (Durapore PVDF, 13 mm diam. 0.22 μ m pore size. Millipore, Billerica, MA) prior to injection. A standard bixin (ChromaDex) was used to generate the calibration curve. Samples were quantified based on their column retention time relative to known bixin standard and absorption spectra of individual peaks.

