

Free Proline

Time required: 3day

- Fully expanded 'sun' leaves from field-grown plants were sampled.
- Purified proline was used to standardize the procedure for quantifying sample values.
- Reagents. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation, until dissolved.
- Kept cool (stored at 4°C) the reagent remains stable 24 hours

Procedure

- A. Approximately 0.5g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered and the Extraction was repeated.
- B. Two ml of the aliquot is mixed with 2 ml of acid ninhydrin followed by 2 ml of glacial acetic acid.
- C. The mixture incubated at 100⁰ C in hot water bath for one hour.
- D. The incubated mixture was transferred to ice water bath to stop the reaction
- E. 4 ml of toluene is added
- F. After shaking the contents thoroughly, the toluene color complex read at 520 nm.