Assay of Acid Phosphatase activity from Potatoes

Aim: To determine the acid phosphatase activity in potatoes using Sodium-β-Glycerophosphate as Substrate

Principle:

Phosphatases liberate inorganic phosphate from organic phosphate ester. Acid phosphatase hydrolases a number of phosphomonoesters and phosphor proteins. Acid phosphatases can be assayed by measuring the amount of inorganic phosphorus released from Sodium-β-Glycerophosphate. The phosphorous released is estimated by Fiske Subbarao method.

Reagents:

1. **Citrate buffer (pH 5.6):**

   Preparation:

   - **1M Citric acid :** Dissolve 5.25 gms of citric acid in distilled water and makeup to 250ml with distilled water
   - **1M trisodium citrate:** Dissolve 14.7 gms of trisodium citrate in distilled water and makeup to 500ml

     →Mix 137ml of citric acid with 363 ml of trisodium citrate solution and set up pH 5.6 buffer solutions.

     →(0.02M Sodium-β-Glycerophosphate in citrate buffer.)

2. **10% TCA** (Tri Chloroacetic acid)

Enzyme Extract:

Clean and wash the potatoes with distilled water and peel out the skin. Weigh about 200gms by using rough balance. Cut it into small pieces then add 200ml of ice cold distilled water and homogenized in a grinder. Filter the homogenate using a cloth and stored the filtrate in a refrigerator.

Procedure:

Take 5ml of Buffer substrate clean and dry the test tube then preincubated for 30 minutes at 37°C. . At the end of the incubation period add 2.5 ml of % TCA and mix well. Keep the test tubes for another minute for room temperature and filter the contents. Take 1ml of the filtrate for the denaturation of inorganic phosphorous by Fiske-Subbarao method

Set up a control simultaneously by adding ml of TCA to 5 ml of buffer substrate followed by ml of enzyme and proceeds as per test.

To determine the protein content of the enzyme extract by biuret method. For this experiment, take 1 ml of extract adds 1 ml of water and add 3ml of biuret reagent and follows as per the biuret estimation procedure.

Report: The amount of inorganic phosphorous present in the given unknown sample is _____ mg of inorganic phosphate formed per 1 ml of enzyme / 30 minutes.

S.No.	Filtrate (ml)	Distilled water (ml)	5N H2SO4 (ml)	Ammonium molybdate (ml)	ANSA (ml)	Distilled water (ml)	A_{640}nm
BLANK	0.5	0.5	1.0	1.0	0.1	6.9	
TEST	0.5	0.5	1.0	1.0	0.1	6.9	

Calculation:

Net OD = Test – Blank

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Filtrate = 8ml in 0.5ml of enzyme from standard Fiske – subbarao graph

_____OD =_____µg of inorganic phosphate formed / 0.5ml of filtrate / 30' of incubation

For 8ml = _____ µg of inorganic phosphate formed / 0.5ml of enzyme / 30 minutes

_____X____X____ µg of inorganic phosphate formed / 1ml of enzyme / 30minutes.

_____ µg of inorganic phosphate formed / 1ml of enzyme / 30minutes

_____mg of inorganic phosphate formed / 1ml of enzyme / 30minutes.