

4. Protein indication (biuret reaction)

4	PROTEIN INDICATION (biuret reaction)	
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The aim of the laboratory practice is to familiarize with the chemical method for determining the protein in natural products.

INTRODUCTION

Proteins are divided into a plurality of groups in the solubility point of view. Albumin dissolves in water, globulin required for the presence of salt ions and glutelin acidic or basic environment.

Biuret reaction is a test that helps detect peptide bonds.

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APPARATUSES AND APPLIANCES

Kitchen heater (cooker)

GLASS AND CHEMICALS

Conical (Erlenmeyer's) flask, glass test tubes,

0.5M solution KCl, 10% solution NaOH, 0.2 M solution NaOH 1% solution CuSO_4 ,

MEASURING SCHEDULE

I. Preparation of substrate

- a. Isolation of globuline. Take the 1 g of powdered bean seed and make an extraction of the protein using 25 ml of 0.5 M solution of KCl in the Erlenmeyer's flask by 15 minutes with shaking on the laboratory shaker. After sedimentation of the powder take the sample of over sediment liquid for further reactions.
- b. Isolation of gluten. Make o dough taking 30 g of wheat flour and water and leave for 15 minutes, proteins absorb water and swells. Next rinse the dough under the stream of cold water pushing it by the spoon (or by hand). Remain part of dough solute in the 30 ml of 0.2 M solution of NaOH.
- c. Take 5 g of nuts (walnuts, hazelnuts or peanuts) and grind them in the mortar. Make an extraction of proteins in the conical flask using 25 ml of 0.5 M solution of KCl by 15 minutes with shaking on the laboratory shaker. After sedimentation of the powder take the sample of over sediment liquid for further reactions.
- d. Take 5 g of seeds (sunflower, cheakpea) and grind them in the mortar. Make an extraction of proteins in the conical flask using 25 ml of 0.5 M solution of KCl by 15 minutes with shaking on the laboratory shaker. After sedimentation of the powder take the sample of over sediment liquid for further reactions.
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II. Reactions

Take the sample of fluids to the test tubes number:

1. egg fluid
2. solution obtained under the isolation of globuline
3. solution obtained under the isolation of gluten
4. solution selected from points c. or d.
5. pure water with some drops of 10% NaOH solution (reference sample).

Introduce some drops of 1% CuSO_4 solution and observe the changes of the solution color. Changes from light-blue to the blue-violet-red indicates the presence of the proteins in the sample.

Notate observations

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Questions (short, several statement answer):

1. What kind of proteins are present in the nuts or seeds?
2. What kind of bond is relate to the biuret reaction?

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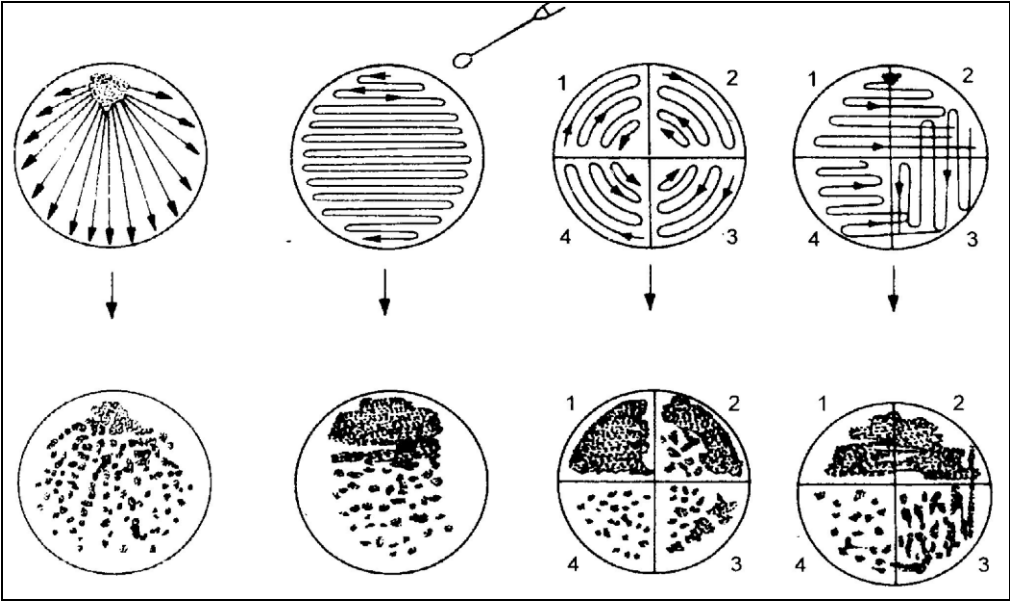


Fig. 1. Ways of inoculum introduction on the surface of agar substrate

First and second way of inoculation is characteristic for the liquid inoculum introduction. Third and fourth is used for inoculation by the hard sample of substrate: gels, waxes, muds or saliva