

## 5. Caffeine identification

5	Caffeine identification	
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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

Caffeine, otherwise teina is a purine alkaloid of the formula

An essential oil is a hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted. The name “essential” comes from the essence of the plant fragrance. In some cases the term “essential” is used for the nutrition “essential amino acids” or “essential fatty acids” which are necessary for live but these terms are not the same in identity to volatile oils.

Volatile oils are generally extracted by distillation, often by using hot water vapor (steam). Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing.

Examples of volatile oils: eugenol from clover rods, limonene from peel of the orange or lemons, cedar wood oil, peppermint oil, thymol from thyme blossom, eucalyptus oil, spearmint oil, etc.

Distillation: organic material, consisting of the flowers, leaves, wood, bark, roots, seeds, or peel, is put into an alembic (distillation apparatus) over water. As the water is heated, the steam passes through the plant material, vaporizing the volatile compounds. The vapors flow through a coil, where they condense back to liquid, which is then collected in the receiving vessel.

Expression: citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus-fruit oils are cheaper than most other essential oils.

Before the discovery of distillation, all essential oils were extracted by pressing.

Solvent extraction: flowers contain too little volatile oil to undergo expression; their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvents are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil-soluble) plant material. For example about 800 kg of rose flakes are used to obtain 1 g of rose oil.

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4	<b>PROTEIN INDICATION (biuret reaction)</b>	
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### APPARATUSES AND APPLIANCES

Kitchen heater (cooker)

### GLASS AND CHEMICALS

Beakers, evaporation glasses, clock glasses, HCL, NaOH, amylase enzyme, iodine solution

### MEASURING SCHEDULE

- I. Preparation of substrate
  - a. cut from the potato the 2-3 mm chips – four samples,
  - b. prepare the solutions of acid (pH=2), base (pH=12) and amylase enzyme (0.5 gram), volume of each is 50 cm<sup>3</sup>,
  - c. introduce chips to the evaporation glasses and flood three samples with adequate solutions,
  - d. sample with enzyme solution should be boiled,
  - e. fourth sample is control one without any action,
  - f. after one hour pour out the solutions (chips from base and acid solution could be washed out with mixed in the aim of neutralization of the chips surface)
  - g. introduce the drop of iodine solution on every chips surface and observe changes by 15 minutes.

### Data procesing

No calculation is necessary

Questions (short, several statement answer):

1. What kind of organic substance starch is?
2. What could be the purpose of starch hydrolysis?

5. Caffeine identification

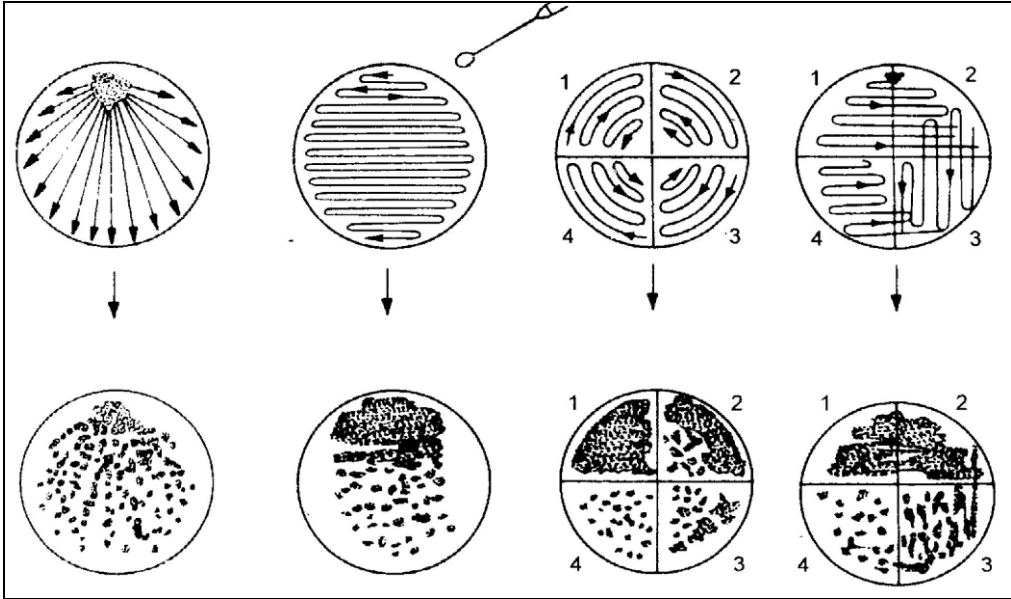


Fig. 1. Ways of inoculum introduction on the surface of agar substrate

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