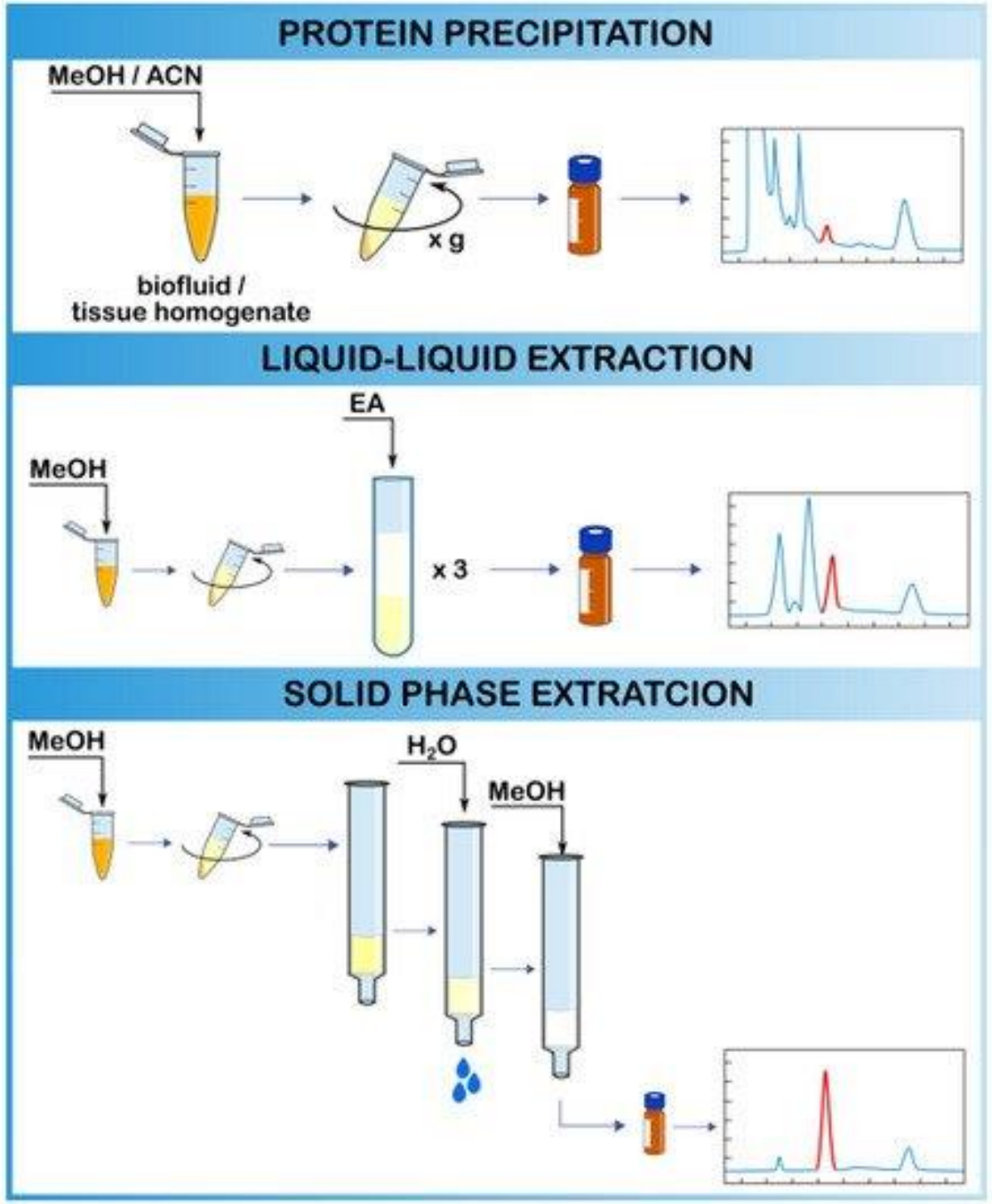
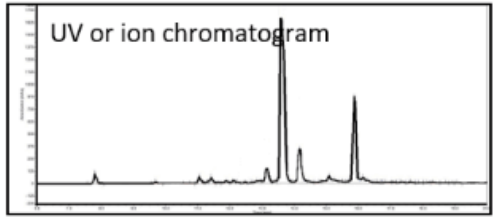

PROTEIN PRECIPITATION
MeOH / ACN
x g
biofluid /
tissue homogenate
LIQUID-LIQUID EXTRACTION
EA
MeOH
x 3
SOLID PHASE EXTRATCION
MeOH
H2O
MeOH

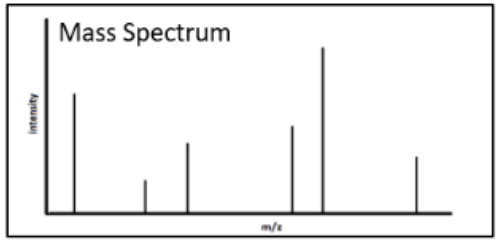
Table 2: Biomolecules purified in ATPS by affinity.

Biomolecule	Ligands attached to PEG	Recovery (%)	Purific. factor	Reference
Lactate dehydrogenase	Tryazine dye-Cibacron Blue F3G-A	81.3	7.4	LIN *et al.* (1998)
β-galactosidase	p-aminophenyl-β-D-thiogalactopiranoside – (APGP)	83	6	SILVA *et al.* (1997)
Protein A	IgG human	87	-	SUZUKI *et al.* (1995)
Lactate dehydrogenase	Eudrogit-Cibacron Blue	54	11.7	GUOQIANG *et al.* (1994)
Penicillin acylase	Trimethylamina	97	25.7	GUAN *et al.* (1992)
Trypsin	Trypsin inhibitor	82	-	LUONG & NGUYEN (1990)

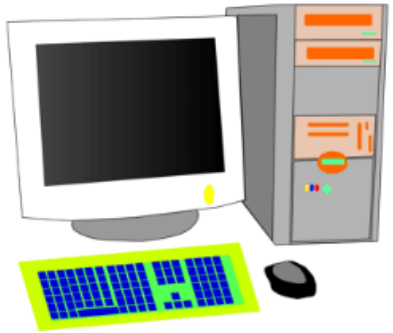
Sample collection

Sample preparation

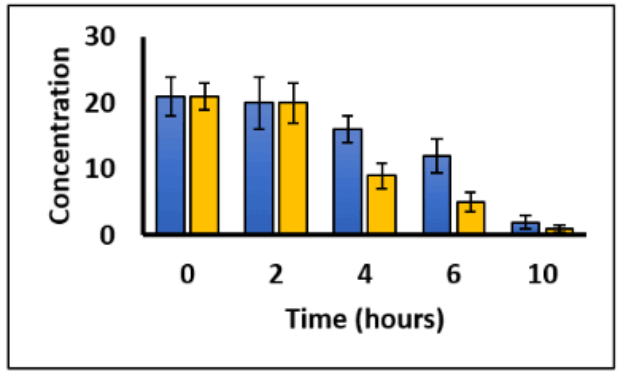




Data acquisition



Data analysis



Results

Sample Preparation

Environmental and Food Analysis

Asst.Prof.Dr.Woravith Chansuvarn

Timeline: Analytical Process

Select method

Sampling / Sample preservation

Sample preparation

Measurement

Evaluation

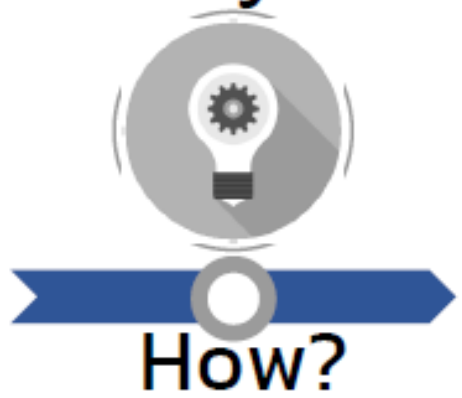
Sample Preparation Perspective

What?

Why?

How?

Sample Preparation



- Standard method
- Protocols
- Validation method
- Limit permission and regulations
- CRM/SRM/TRM

Sample Preparation Perspective

Sample preparation is a process required for the transformation of a sample to make it amenable for chemical analysis or to improve the analysis.

The major goal of sample preparation is **to prepare the sample for the separation/ detection part of the analysis**.

Sample preparation is the way in which a sample is treated to prepare for analysis. Sample preparation is carefully critical in analytical chemistry to accurately generate either a standard or unknown sample for a chemical measurement.

> Sample preparation is common in many analyses and is developed to allow or to improve a specific analysis. This step may be the most time-consuming in an analysis and affects significantly the analytical information.
>
> S.C. Moldoveanu, V. David, Journal of Chromatography Library, 2002

The Ultimate Goals

- Transform analyte from a non-compatible environment into the instrumental compatible with analytical techniques
- Remove unwanted matrix components that may interfere with the analysis of the desired compound
- Improve limits of detection and/or quantitation (Enrichment analyte concentration)
- Separate/isolate of individual components from complex mixtures or matrix

Standard method

International standard

Regional standard

National standard

Association standard

Society standard

Publication

Developing method

Standard method

วิธีมาตรฐานระดับนานาชาติ (International standard)

เป็นมาตรฐานที่ได้จากข้อตกลงร่วมกันของประเทศสมาชิกต่างๆ ที่มีความสนใจร่วมกัน

- วิธีมาตรฐาน ISO (International Standards Organization)

ISO International Organization for Standardization

ISO/TS 15495 | IDF/RM 230:2010, Milk, milk products and infant formulae-Guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS

Standard method

วิธีมาตรฐานระดับภูมิภาค (Regional standard)

เป็นมาตรฐานที่เกิดขึ้นจากการประชุม ปรึกษาหารือกันระหว่างประเทศในภูมิภาคเดียวกัน

- วิธีมาตรฐานของสหภาพยุโรป (European standard)

EN 1233:1996 Water quality–Determination of chromium–Atomic absorption spectrometric methods

EN 14084:2003 Foodstuffs–Determination of trace elements–Determination of lead, cadmium, zinc, copper and iron by AAS after microwave digestion

Standard method

วิธีมาตรฐานระดับประเทศ (National standard)

เป็นมาตรฐานที่ได้จากการประชุมหารือเพื่อหาข้อตกลงร่วมกันของผู้เกี่ยวข้องหลายภาคส่วนในประเทศ

- Japanese Industrial Standard (JIS)
- British Standard (BS)
- USEPA
- APHA
- AWWA
- NIOSH
- OSHA
- มอก.

Standard method

วิธีมาตรฐานระดับสมาคม (Association standard)

เป็นมาตรฐานที่กำหนดขึ้นจากกลุ่มบริษัทที่อยู่ในวงการค้าเดียวกัน หรือเกิดจากข้อตกลงของกลุ่มบริษัทหรือโรงงานที่มีกิจกรรมของอุตสาหกรรมเป็นอย่างเดียวกัน หรือมีการผลิตของชนิดเดียวกัน

- AATCC (American Association of Textile Chemists and Colorists)

AATCC 112:2014

Formaldehyde release from fabric, Determination of: sealed Jar method

Standard method

วิธีมาตรฐานรับรองโดยองค์กรทางวิชาการ

ปัจจุบันมีหลายองค์กรที่เป็นที่ยอมรับในระดับนานาชาติ

- ASTM
- AOAC
- AOCS
- APPA
- AWWA
- EPA

ASTM D5630–13 Standard test method for ash content in plastics

AOAC Official Method 999.10 lead, copper, zinc and iron in foods: Atomic absorption spectrometry after microwave digestion

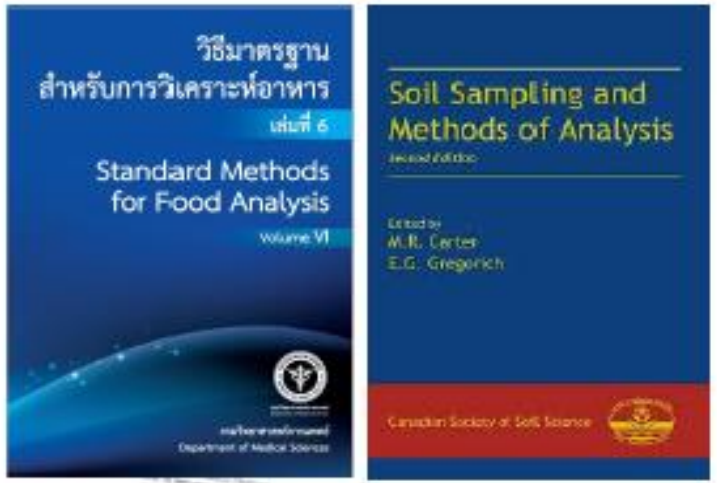
AOCS official method Ca 12-55 Phosphorus

Standard method

วิธีเผยแพร่ในตำรา/วารสารวิจัย (Publication method)

ส่วนใหญ่เป็นวิธีที่พัฒนาขึ้นใหม่

- วิธีมาตรฐานสำหรับการวิเคราะห์อาหาร โดยกรมวิทยาศาสตร์การแพทย์
- Analytical Chemistry
- Journal of Chromatography A
- Analytica Chimica Acta
- Food Chemistry
- Analyst
- Microchimica Acta
- Analytical Letters



AOAC Official Method 931.08 Formaldehyde in Food

First Action 1931

(See also 964.21 [see 44.5.14].)

UV-Vis method

A. Preparation of Test Portion

If food is solid or semisolid, macerate 100 g with 100 mL H_2O in mortar. Transfer to 800 mL Kjeldahl flask, acidify with H_3PO_4, add 1 mL excess, connect with condenser through trap, and slowly distil 50 mL. For milk, dilute 100 mL with 100 mL H_2O, and acidify and distil as for solids. With other liquid foods, acidify 200 mL and distil as for solids.

B. Chromotropic Acid Test

(a) Reagent.—Prepare saturated solution of 1,8-dihydroxynaphthalene-3,6-disulfonic acid (ca 500 mg/100 mL) in ca 72% H_2SO_4 (pour 150 mL H_2SO_4 into 100 mL H_2O and cool). Solution is light straw-colored.

(b) Test.—Place 5 mL reagent in test tube and add, with mixing, 1 mL distillate, A. Place in boiling H_2O bath 15 min, and observe during heating period. Presence of HCHO is indicated by appearance of light to deep purple (depth of color depending on amount of HCHO present).

Reference: Z. Anal. Chem. 110, 22(1937).

Source : http://files.foodmate.com/2013/files_2990.html

Food Chemistry 202 (2016) 476–483

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of formaldehyde in food and feed by an in-house validated HPLC method

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[a] National Food Safety Laboratory, Institute of Public Health, Mohakhali, Dhaka, Bangladesh
[b] Food and Agriculture Organization of the United Nations, Dhanmondi, Dhaka, Bangladesh

HPLC method

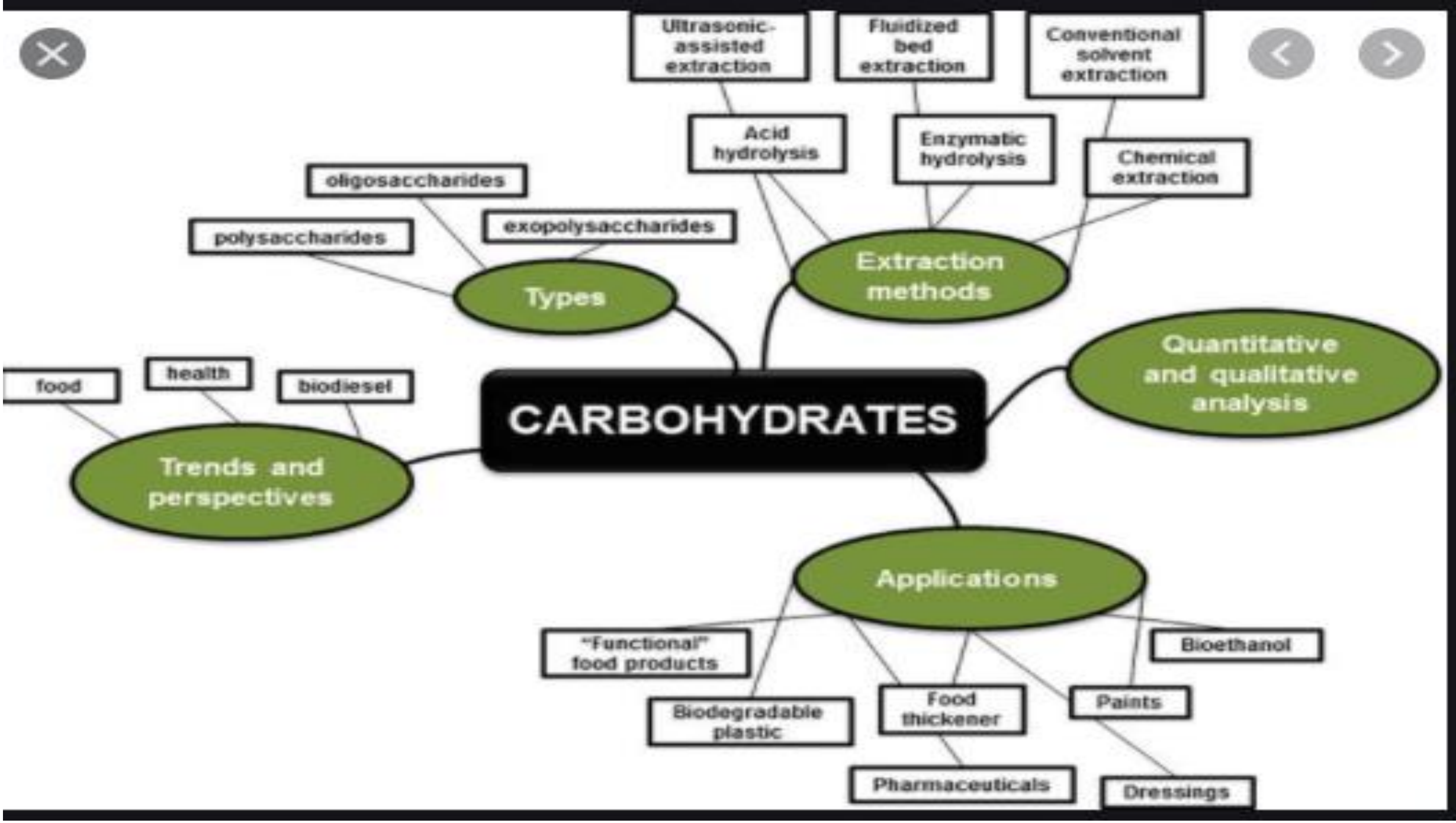
Derivatization kinetics followed the procedure described by Claeys et al. (2009) but was slightly modified. Edible parts of the food; fruit flesh and fish fillets were used for the analysis. For derivatization kinetics, mango samples were ground, homogenized and spiked with 10 mg/L of formaldehyde standard. To sample aliquots of 5 g, 5 mL of acetonitrile were added, and the sample vortexed and then sonicated for 30 min. The samples were centrifuged at 5000 rpm for 5 min and the supernatant was passed through a 90 mm diameter Whatman® 541 (Hardened Ashless) filter paper (SIGMA–Aldrich, Buchs SG, Switzerland). Two and half milliliter of 2,4 DNPH was added to the extract and mixed well. Samples were incubated at 40 °C for 30, 60, 90 and 120 min in a shaking water bath (model BS-11, Oxon, UK). Formaldehyde was quantitatively converted to its Schiff base in 60 min. In all experiments, derivatization time was set to 60 min. After incubation, the acetonitrile layer was collected, membrane filtered (0.45 μm) and injected into the HPLC.

Lipid preparation and extraction

The lipid extraction by Folch method

- Homogenized by using 2:1 chloroform/methanol mixture
- Filltration by (fillter paper or centrifuge)
- Washing with (water or NaCl solution)
- The mixture is centrifuge
- Remove the upper phase (Methanol) by siphoning
- The lower phase (Chloroform)
- **Result: LIPIDS**

Carbohydrate preparation and extraction



REACTIONS OF SUCROSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Sucrose is a carbohydrate
2)	Benedict's Test	No color change	It is a non reducing carbohydrate
3)	Barfoed's test	No change in color	It is not a mono saccharide
4)	Seliwanoff test	Cherry red color	Keto hexose containing disaccharide
5)	Hydrolysis (Inversion) test	The hydrolytic products give positive reaction with Benedict's and Barfoed's reagents.	Confirmatory test for Sucrose
6)	Osazone test	No reaction	Sucrose does not form osazone crystals

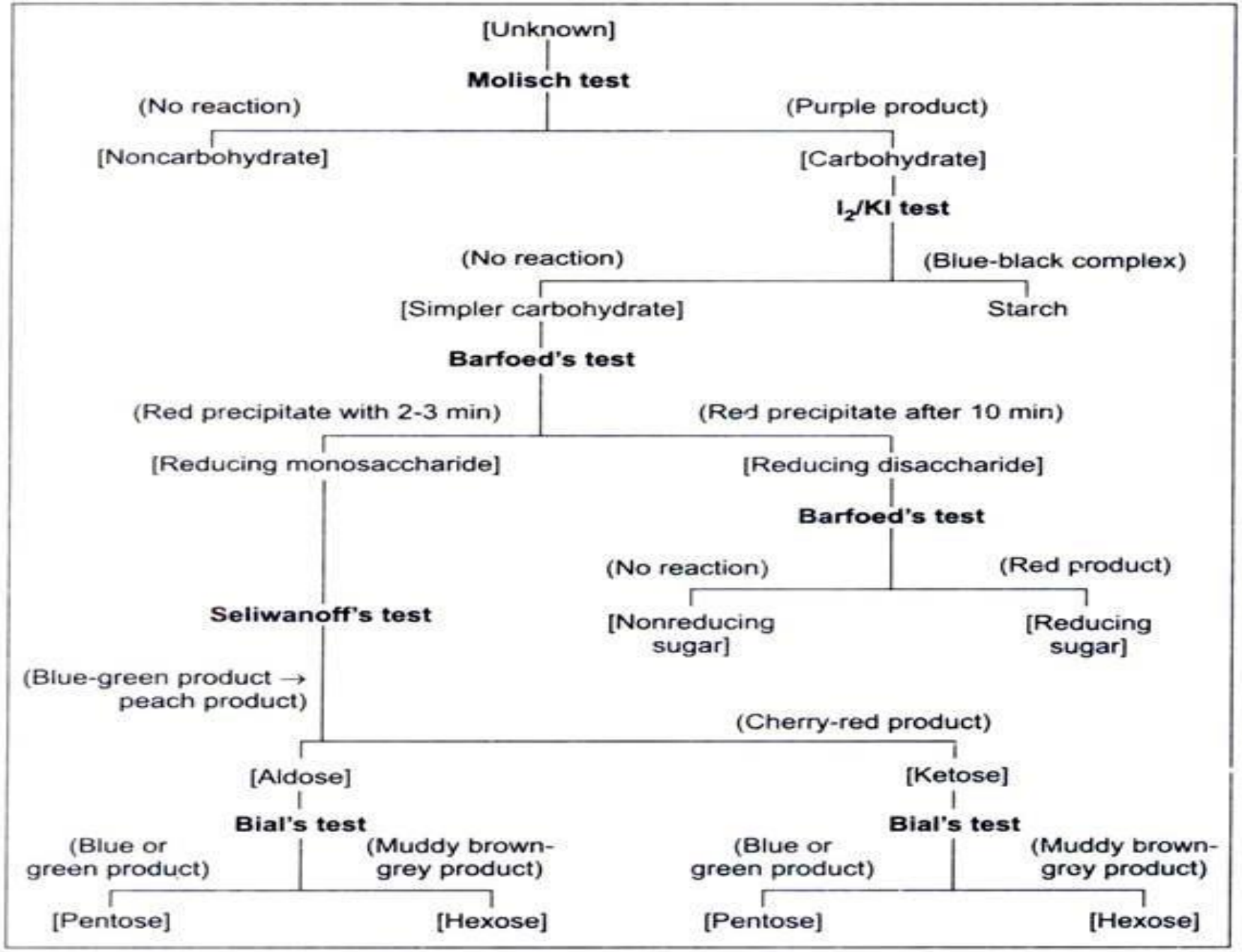
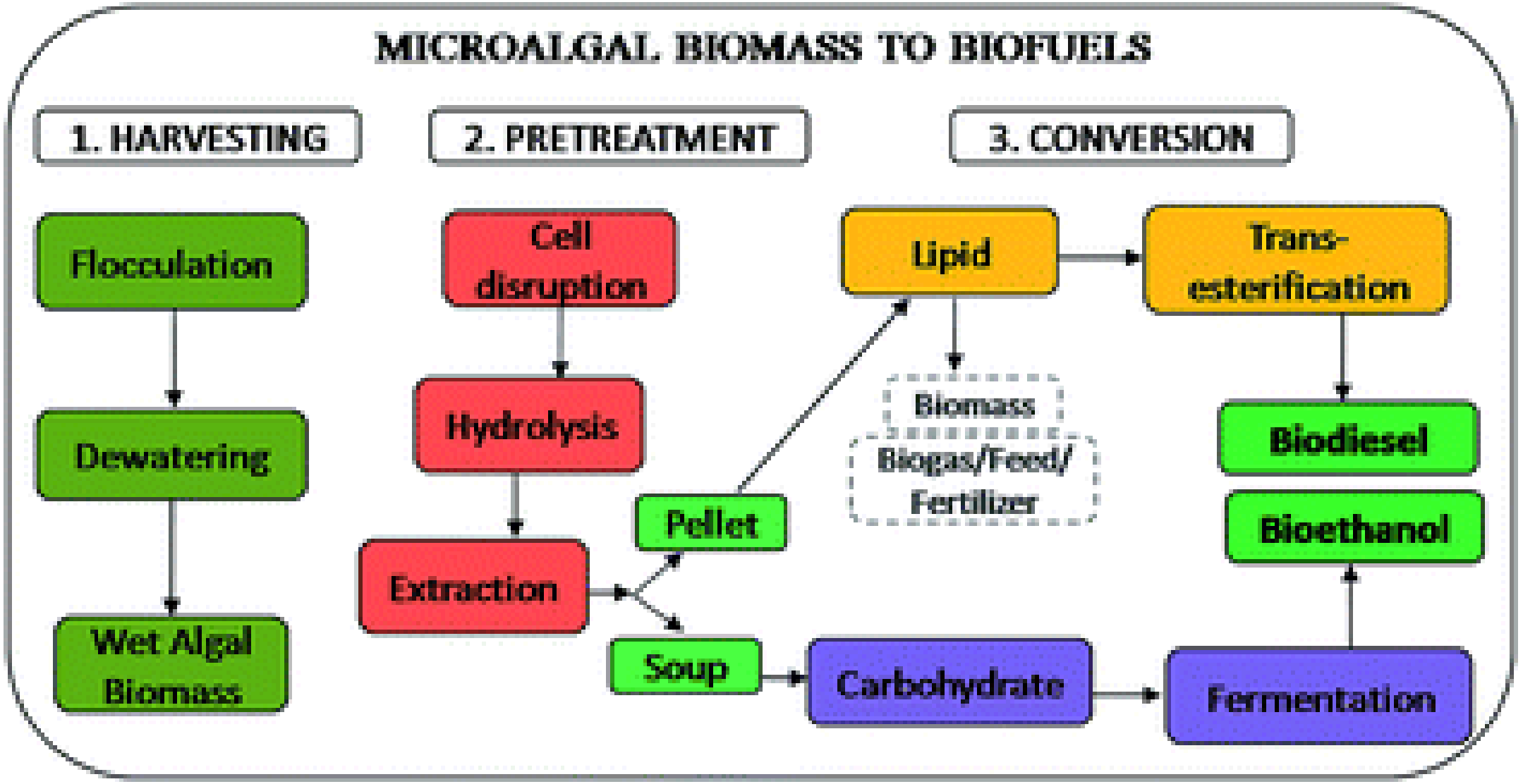
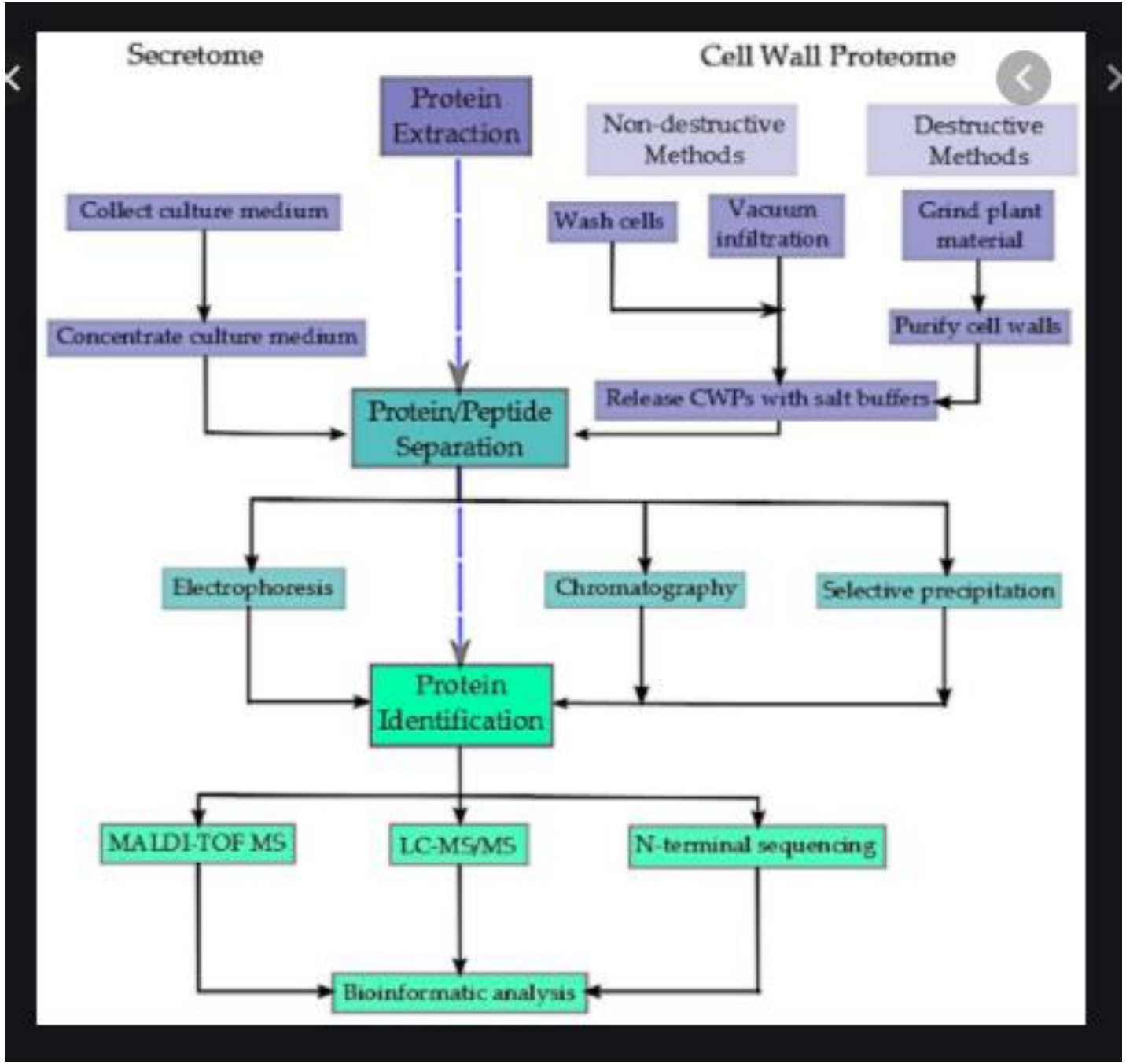


Fig. 18.1: Flow chart for classifying unknown carbohydrate

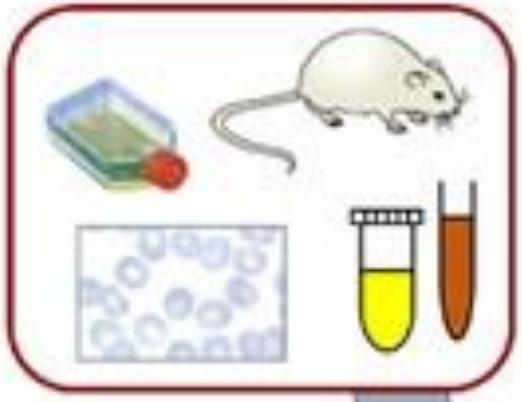


Protein preparation and extraction



Animals

Identification of modulated proteins /PTMS
Identification of new interacting partners
....



Cell lysis/subcellular fractionation

Protein extraction

Protein enrichment/ fractionation

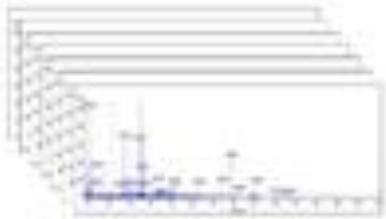
Enzymatic digestion

Peptide enrichment/ fractionation

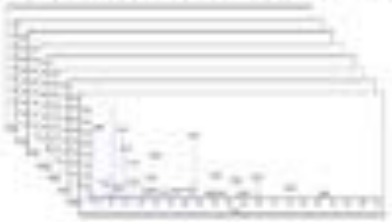
nanoLC-column

MS
MS/MS

TopN Data-dependent analysis

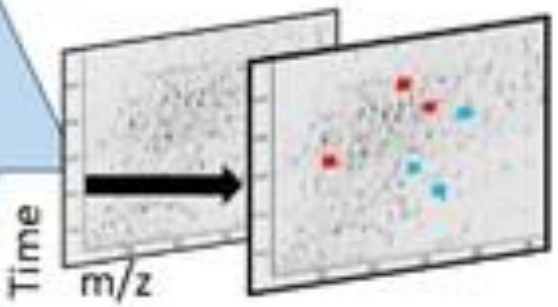


MS scans
(~ 50 000 scans/ analysis)



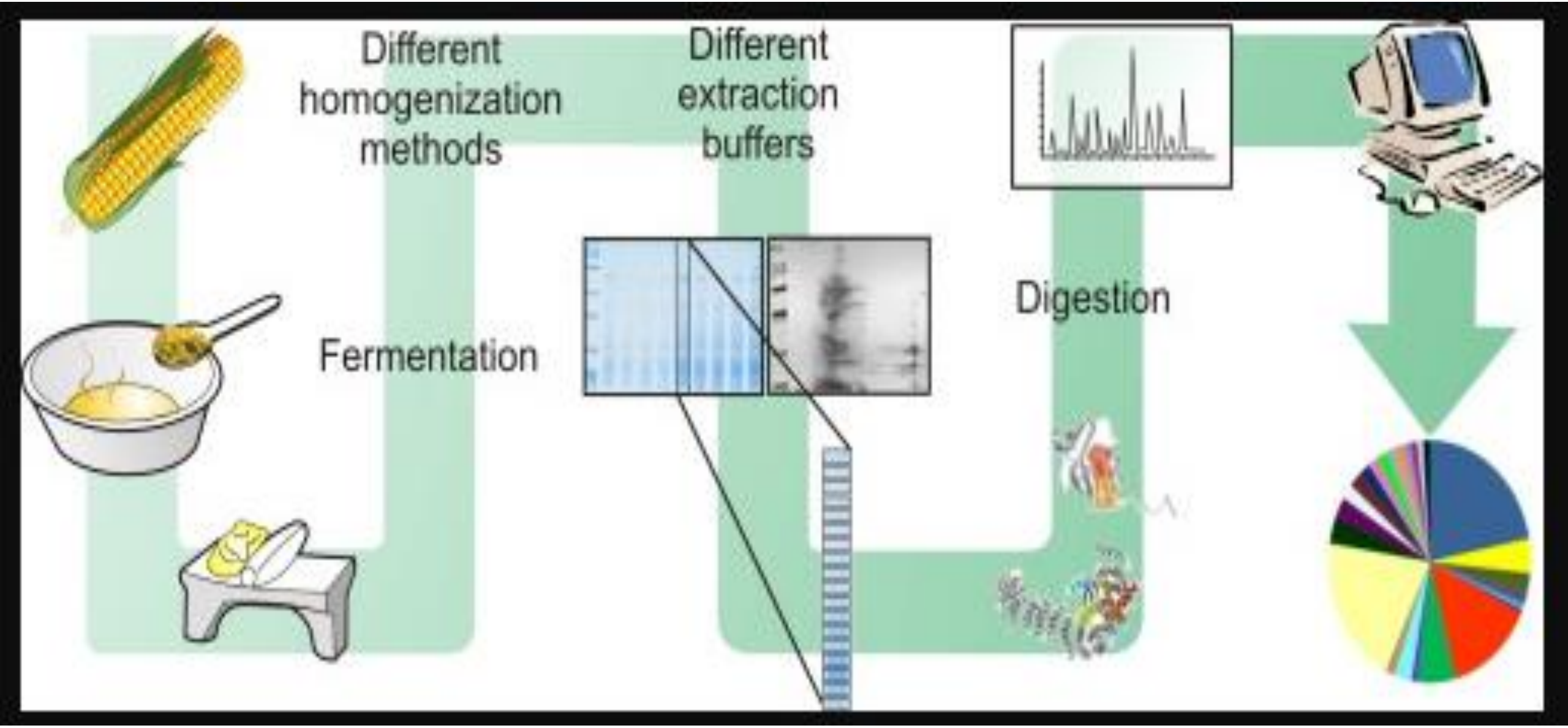
MS/MS scans
(~30 000 spectra / analysis)

Bioinformatic processing

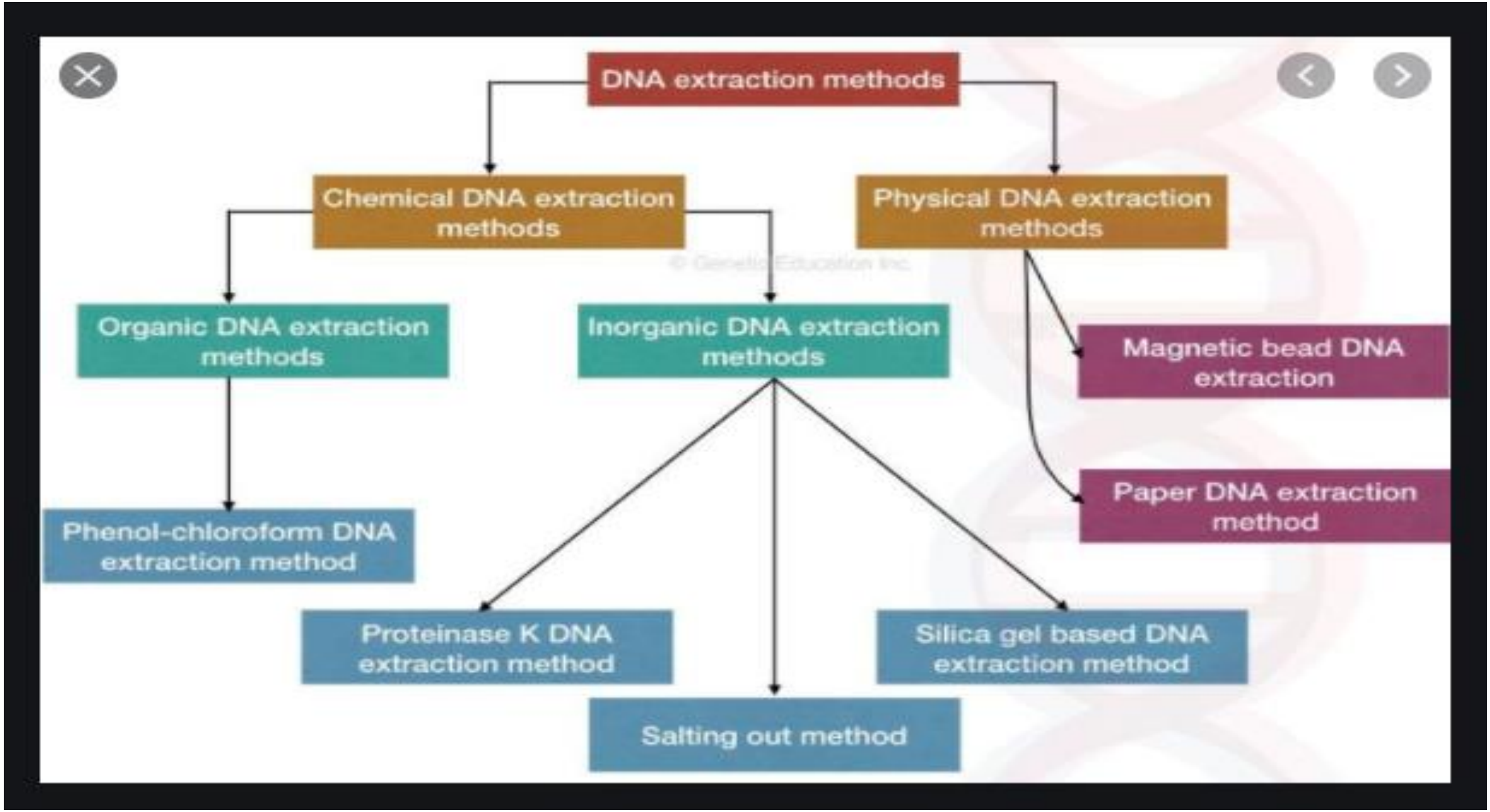


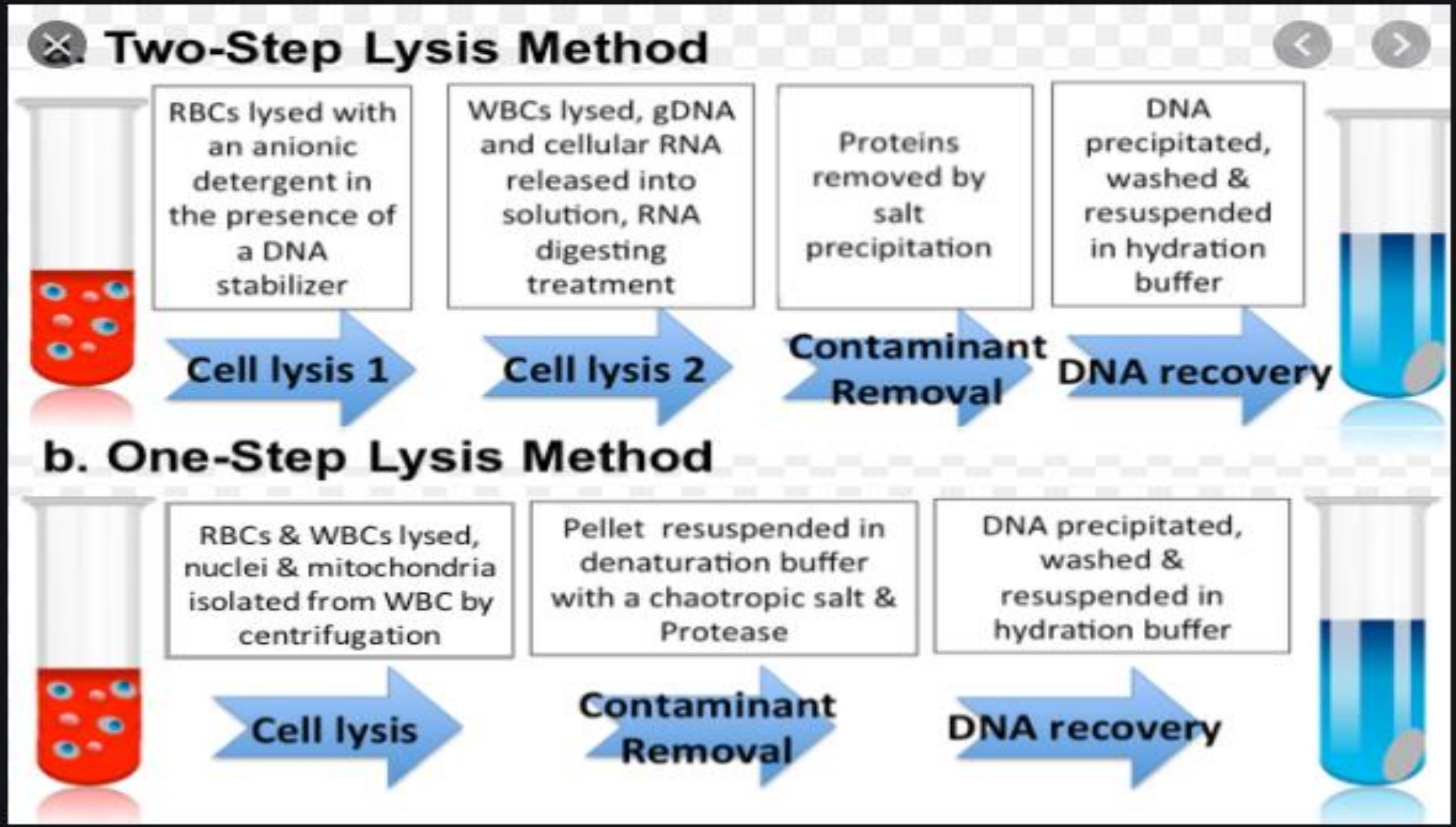
Statistical analysis

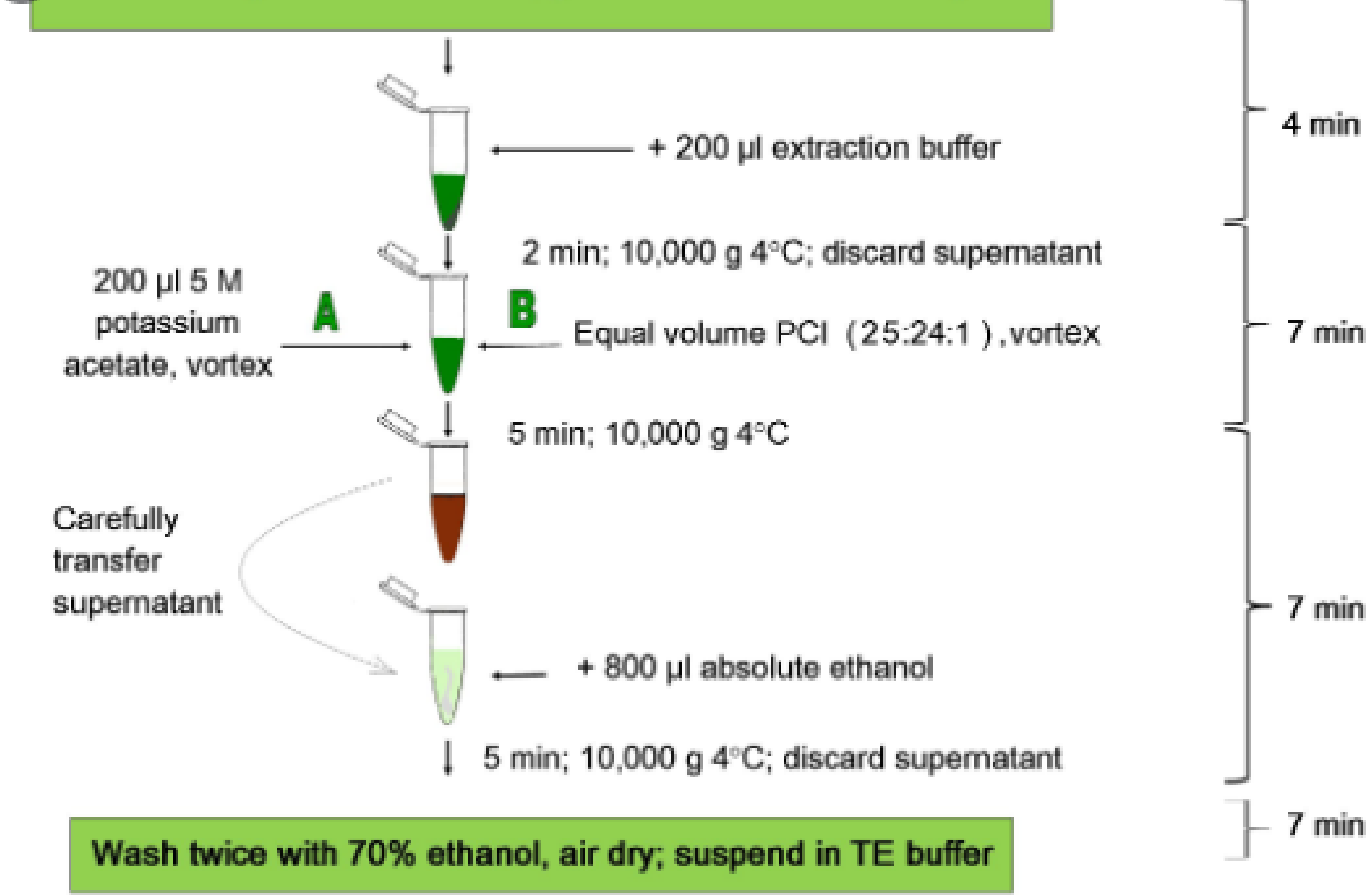
Plants


Different homogenization methods
Different extraction buffers
Digestion
Fermentation

Nucleic acid preparation and extraction


DNA extraction methods
Chemical DNA extraction methods
Physical DNA extraction methods
Organic DNA extraction methods
Inorganic DNA extraction methods
Magnetic bead DNA extraction
Paper DNA extraction method
Phenol-chloroform DNA extraction method
Proteinase K DNA extraction method
Silica gel based DNA extraction method
Salting out method


a. Two-Step Lysis Method
RBCs lysed with an anionic detergent in the presence of a DNA stabilizer
WBCs lysed, gDNA and cellular RNA released into solution, RNA digesting treatment
Proteins removed by salt precipitation
DNA precipitated, washed & resuspended in hydration buffer
Cell lysis 1
Cell lysis 2
Contaminant Removal
DNA recovery
b. One-Step Lysis Method
RBCs & WBCs lysed, nuclei & mitochondria isolated from WBC by centrifugation
Pellet resuspended in denaturation buffer with a chaotropic salt & Protease
DNA precipitated, washed & resuspended in hydration buffer
Cell lysis
Contaminant Removal
DNA recovery


+ 200 µl extraction buffer
4 min
2 min; 10,000 g 4°C; discard supernatant
200 µl 5 M potassium acetate, vortex
A
B
Equal volume PCI (25:24:1), vortex
7 min
5 min; 10,000 g 4°C
Carefully transfer supernatant
7 min
+ 800 µl absolute ethanol
5 min; 10,000 g 4°C; discard supernatant
7 min
Wash twice with 70% ethanol, air dry; suspend in TE buffer

The Ends