2. Lab Safety

By the end of this briefing, participants will be able to:

- Identify key hazards in laboratories (chemical, biological, physical, electrical).
- Apply core safety practices: PPE, hygiene, labeling, storage, and waste disposal.
- Respond appropriately to spills, exposures, fires, and injuries.
- Locate and correctly use emergency equipment and safety data resources.

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1) Laboratory Rules and Responsibilities

- Authorization only: Work in the lab only after training and supervisor approval.
- No food, drink, smoking, or cosmetics. Tie back long hair; avoid loose clothing.
- Access control: Keep doors closed; do not prop emergency exits.
- Housekeeping: Keep benches uncluttered; clean as you go; keep floors dry and clear.
- Signage: Heed hazard pictograms and equipment tags (in/out of service).
- Never work alone with hazardous operations; use a buddy or inform a colleague.

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2) Personal Protective Equipment (PPE)

- Minimum PPE: Lab coat, safety glasses/goggles, long pants, closed-toe shoes, appropriate gloves (must match to hazard)
- PPE use: Inspect before use, remove before leaving lab areas, never reuse disposable gloves, wash hands after removal.

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3) Chemical Safety Essentials

- Before use: Read the Safety Data Sheet (SDS), check incompatibilities, exposure limits, and first aid.
- Fume hoods: Use for volatile, toxic, or malodorous substances; keep sash low; maintain 10–15 cm (4–6") setback from the sash; minimize clutter.
- Measuring and transfer: Use secondary containment for transport; use proper funnels and pipettes; never mouth pipette.

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4) Biological and Sharps Safety

- Biosafety levels: Follow designated practices decontaminate work surfaces before/after.
- Aseptic technique: Limit aerosols; use biosafety cabinets for procedures with splash/aerosol risk.
- Sharps: Use safety-engineered devices when possible; never recap needles; dispose of sharps immediately in approved containers.
- Bio-waste: Collect in labeled, autoclave-safe bags/containers; inactivate as required before disposal.

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5) Physical, Electrical, and Equipment Safety

- Equipment training: Operate only after instruction; follow SOPs; check guards/interlocks.
- Centrifuges: Balance loads; inspect rotors; wait for full stop before opening.
- Electrical: Inspect cords; keep liquids away; lockout/tagout for maintenance; do not use damaged equipment.

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6) Waste Management and Environmental Controls

- Segregation:
    - Chemical waste: By compatibility (halogenated vs. non-halogenated solvents; acids/bases).

  - Biohazard waste: Red bags/approved containers; decontaminate per policy.
  - Sharps: Dedicated puncture-resistant containers only.
  - Glass: Clean, non-contaminated glass to designated bins; contaminated glass follows hazard class.
- Labeling: “Hazardous Waste,” constituents, approximate percentages
- Storage: Closed containers, secondary containment, within designated accumulation areas.
- Do not pour chemicals down the drain unless explicitly allowed by local policy.

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7) Emergency Preparedness and Response

- Know locations: Exits, fire alarms, extinguishers (class/type), safety showers, eyewash stations, spill kits, first aid kits, SDS access.
- Fires:
  - If small and trained: Use PASS method (Pull, Aim, Squeeze, Sweep) with correct extinguisher.
  - Otherwise: Activate alarm, evacuate, close doors, meet at assembly point, report.
- Spills:
  - Chemical: Alert area; don PPE; consult SDS; use spill kit; neutralize acids/bases where appropriate; contain and collect waste properly; report.
  - Biological: Evacuate if aerosol generated; wait appropriate contact time after disinfectant application; clean per biosafety SOP.
  - Mercury/other special spills: Use dedicated kits; never use household vacuums.
- Exposures:
  - Skin: Remove contaminated clothing; flush with water ≥15 minutes; seek medical attention; bring SDS.
  - Eyes: Eyewash rinse ≥15 minutes; hold eyelids open; seek medical attention.
  - Inhalation/ingestion: Move to fresh air; call emergency services; follow medical guidance.
- Incident reporting: Report all incidents, near-misses, and injuries to your supervisor and safety office.

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8) Good Laboratory Practice (GLP) and Documentation

- Planning: Risk assessment before experiments; define controls and emergency steps.
- Records: Maintain lab notebook with procedures, reagents, lot numbers, and deviations.
- Calibration and maintenance: Log equipment checks; quarantine faulty devices with “Do Not Use” tags.
- Sample labeling and chain of custody where applicable.

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9) Conduct and Culture of Safety

- Speak up: Stop work if it is or you feel unsafe; challenge unsafe practices respectfully.
- Mentorship: Senior staff model proper behavior; new staff shadow until competent.

3. Safety check

The following diagram shows several examples of improper behavior in the laboratory.

Identify all the errors and explain the hazards resulting by each instance of improper behavior.

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Protein Detection: Biuret Reaction —
Comparison of Cow's Milk, Soy Milk, Oat Milk, and Albumin (Photometric Analysis)

Objective
The qualitative and quantitative detection of proteins in various beverages using the Biuret reaction and the comparison of protein concentrations via photometric measurement (λ = 540–560 nm).

Chemicals and Substances Used
- Copper sulfate solution ($CuSO_4 \cdot 5H_2O$) 1.0% (w/v) in water
- Sodium hydroxide solution (NaOH) 10% (w/v) in water (alkaline medium for biuret)
- Sodium carbonate solution (Na_2CO_3) 2.0% (w/v) — as an alternative for stabilizing the alkaline environment
- Albumin standard (e.g., bovine serum albumin, BSA) stock solution 1.0 g/L (1.0 mg/mL) in deionized water
- Samples:
  - Fresh cow's milk (unsweetened)
  - Soy milk (unsweetened, preferably without additives)
  - Oat milk (unsweetened, without sweeteners/stabilizers if possible)
- Distilled water (blank sample)

Safety and Disposal Quick Guide (read before starting)
- Personal protective equipment: lab coat, safety goggles, nitrile gloves.
- NaOH is caustic: avoid contact with eyes and skin. In case of contact, rinse immediately with plenty of water.
- Copper sulfate is toxic to aquatic organisms — collect waste separately.
- Immediately neutralize/vacuum up spilled material and dispose of it in the designated chemical waste containers.
- Avoid large quantities of NaOH and copper sulfate; small preparation batches are sufficient for the experiment.
- Do not consume samples (food).
- Dispose of all waste according to regulations; follow the instructions of the local laboratory management!

Equipment and Glassware (per group for 4 samples + calibration series)
- Spectrophotometer (UV/Vis) with cuvettes (1 cm)
- Measuring flasks 10 mL, 50 mL; Erlenmeyer flasks 100–250 mL
- Pipettes (P20, P200, P1000) with matching tips
- Centrifuge (optional, if samples are highly turbid)
- Vortex mixer/stirrer, thermometer, balance
- Test tubes / 15 mL centrifuge tubes

Preparation of reagents (one-time, if not already available)
1. Copper sulfate 1.0% (w/v): Dissolve 1.0 g $CuSO_4 \cdot 5H_2O$ in 100 mL distilled water. Stir well.
2. NaOH 10% (w/v): Slowly dissolve 10 g of NaOH pellets in approximately 70 mL of distilled water (highly exothermic), make up to 100 mL; allow to cool.
3. Na_2CO_3 2.0% (w/v): Dissolve 2.0 g of Na_2CO_3 in 100 mL of water.
4. Biuret Reagent (simplified, proven version for schools):

Mix in a graduated cylinder before use: 25 mL NaOH 10% + 0.5 mL $CuSO_4$ 1% + 4.5 mL distilled water → total 30 mL. Mix well; prepare fresh or store in a cool place (max. a few days).

4. Protein detection

Calibration Series (BSA) — Exact Quantities

Prepare the following standards in 10 mL volumetric flasks using the BSA stock solution (1.0 mg/mL):

samples for calibration	absorbance
Standard 0 (Blank): 0.0 mL BSA + 10.0 mL distilled water → 0.00 mg/mL	
Standard 1: 0.5 mL BSA + 9.5 mL H_2O → 0.050 mg/mL	
Standard 2: 1.0 mL BSA + 9.0 mL H_2O → 0.100 mg/mL	
Standard 3: 2.0 mL BSA + 8.0 mL H_2O → 0.200 mg/mL	
Standard 4: 4.0 mL BSA + 6.0 mL H_2O → 0.400 mg/mL	

(Select other ranges if necessary; these values are suitable for sensitive photometers.)

Sample Preparation (Dilution)

Due to varying protein contents, the beverages are standardized/diluted prior to the reaction. Suggestion:

- Cow's milk: Target concentration in the reaction mixture ≈ 0.1–0.2 mg/mL protein → Dilution 1:100 to 1:200 depending on the estimated protein content (Cow's milk typically ~3.3 g·100 mL−1 → 33 mg/mL).
  Example: 1:200 → 50 µL milk + 9.95 mL distilled H_2O → 0.165 mg/mL (if starting concentration is 33 mg/mL)

- Soy milk: Protein content varies (e.g., 2–3 g·100 mL−1 → 20–30 mg/mL) → Dilution 1:100 to 1:200.
  Example 1:100 → 100 µL soy milk + 9.9 mL H_2O

- Oat milk: Protein content often very low (0.5–1.5 g·100 mL−1 → 5–15 mg/mL) → Dilution 1:20 to 1:50.
  Example 1:50 → 200 µL oat milk + 9.8 mL H_2O

  Albumin (BSA) for verification: Use the standards prepared above directly.

Tip: Perform a preliminary test with a small sample to determine the optimal dilution so that the measured values fall within the range of the calibration curve (0.05–0.40 mg/mL).

Procedure — Detailed Steps (per Sample / Standard)

1. Label reaction vessels/cuvettes for the blank, standards, and samples.
2. Pipette the following into a test tube or 15 mL tube:
   - 1.0 mL diluted sample (or standard)
   - 1.0 mL biuret reagent (see above)
   - Note: Sample:reagent ratio = 1:1 for a good reaction; for other protocols, a 2:1 ratio may be used; adjust reagent volumes accordingly.
3. Mix gently (vortex for 2–3 s) and incubate for 10 min (max. 30 min) at room temperature. A violet color will develop.
4. After incubation:
   - If highly turbid, centrifuge briefly (1–2 min at 2000–3000 rpm) and measure the supernatant (upper liquid layer)
   - Transfer 1.0 mL to a clean cuvette (1 cm) and measure the absorbance at λ = 540–560 nm (540 nm is recommended for typical biuret variants). Set the zero point using a blank (0 mg/mL).
5. Record the absorbances for the blank, standards, and samples.

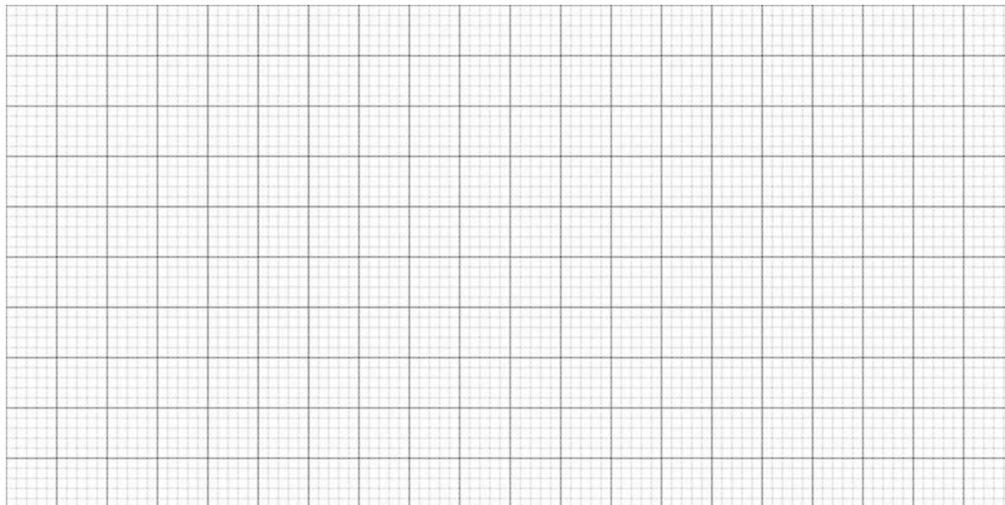
Note: If your photometer only supports 595 nm or 620 nm (typical for Bradford), it is best to select 540–560 nm for Biuret. Consistency is more important than exact wavelength when calibration is performed internally.

Analysis

1. Plot a calibration curve: absorbance (y) versus protein concentration ($mg \cdot mL^{-1}$) of the standards.
2. Calculate the protein concentration of the samples using the calibration curve (linear regression).
3. Correct for the dilution of the original sample to report the protein content in $mg \cdot mL^{-1}$ or $g \cdot 100\ mL^{-1}$.
4. Sample calculation:

o Measured concentration from calibration curve: 0.120 $mg \cdot mL^{-1}$ (in reaction mixture)

- Dilution of the original sample: 1:100 → Original protein content = 0.120 mg/mL × 100 = 12 mg/mL = 1.2 $g \cdot 100\ mL^{-1}$



Brief observation (as expected)

- Blank: no color (very low absorption).
- Albumin standards: Violet color intensity proportional to concentration.
- Cow's milk: Distinct violet color at moderate dilution → high absorption.
- Soy milk: Also violet, intensity comparable to or slightly lower than cow's milk (depending on the brand).
- Oat milk: Very faint to barely detectable violet color at high dilutions; possibly lower absorption at the selected dilution — therefore, choose a lower dilution.

Disposal

- ✓ All used solutions (biuret solution with copper) contain copper and are classified as aqueous waste containing heavy metals.
- ✓ Solid waste (filter paper, pipette tips, etc.) goes in the waste bin.
- ✓ So not neutralize or dilute waste containing copper on your own without consulting the person in charge.
- ✓ Seal reagent bottles tightly, label them, and store them properly.

Tofu Production via Calcium Coagulation —
Comparison of Cow's Milk, Soy Milk, Oat Drink, and Powder

Objective
To produce a protein-based gel ("tofu"/curd) through coagulation with calcium ions and to compare the coagulability of different matrices. To investigate yield, texture, and the effect of calcium dosage. Every group gets one of the four samples.

Chemicals and Materials Used
- Samples:
    - Soy milk, unsweetened (≥2% protein), 1.0 L per group
    - Cow's milk, fresh (3.5% fat), 1.0 L per group
    - Oat drink, unsweetened ("clean label," preferably without thickeners), 1.0 L per group
    - Mix protein powder with distilled water according to instructions
- Coagulants (select one variant or both for comparison):
    - Calcium chloride solution 10% (w/v) → Dilute 10 g $CaCl_2 \cdot 2H_2O$ to 100 mL with distilled water
    - Calcium sulfate suspension 2% (w/v) → Disperse 2 g $CaSO_4 \cdot 2H_2O$ thoroughly in 100 mL of warm water (Remains as a suspension)

Safety and Disposal Quick Guide (read before starting)
Safety/Note: $CaCl_2$ is hygroscopic/irritating; $CaSO_4$ is food-safe.
Work with gloves and goggles. Be careful of hot medium.

Equipment and Materials
- Hot plate with stirring capability or water bath
- Thermometer (0–100 °C), pH paper, or pH meter
- Beakers (2 × 1 L) or pots, stirring spoon or stir bar
- Sieve, filter cloth or cheesecloth, tofu/cheese mold (optional)
- Scale, measuring cylinder, beakers (50–250 mL)
- Press or small weight (0.5–2 kg)
- Timer, labeling supplies, bowls for whey

Procedure
For each matrix, three increasing doses are added. Recommended steps (proceed similarly for $CaSO_4 \cdot 2H_2O$):
- For soy milk:
  1.5 g, 2.0 g, 2.5 g $CaCl_2 \cdot 2H_2O$ per liter (15, 20, 25 mL of a 10% solution)
- For cow's milk:
  0.5 g, 1.0 g, 1.5 g $CaCl_2 \cdot 2H_2O$ per liter (5, 10, 15 mL of 10% solution)
- For oat milk:
  1.5 g, 2.5 g, 3.5 g $CaCl_2 \cdot 2H_2O$ per liter (15, 25, 35 mL 10% solution); realistically, no stable gel formation is to be expected; this serves to demonstrate the low coagulability
- For protein powder/drink:

5. Tofu production

1.5 g, 2.0 g, 2.5 g $CaCl_2 \cdot 2H_2O$ per liter (15, 20, 25 mL 10% solution)

Work separately for each group one sample (0.5 L):

1. **Heat while stirring**
   - Soy milk: Heat to 82–85 °C and maintain for 2 minutes.
   - Cow's milk: Heat to 35–40 °C (this temperature is sufficient for pure calcium-induced casein coagulation).
   - Oat drink: Heat to 80–85 °C as with soy milk (if stabilizers are present, this may further complicate coagulation).
   - Protein drink: Heat to 35–40 °C (to prevent coagulation caused by possible additives).
2. **Addition of the coagulant**
   - Turn off the heat. Note the target temperature when adding.
   - Slowly add the calculated amount of 10% $CaCl_2$ solution (or corresponding $CaSO_4$ suspension) in 2–3 portions. Stir gently (no strong shear), wait 30–60 seconds after each portion.
   - After the final addition, stop stirring and let stand for 10–15 minutes.
3. **Separation**
   - Visual inspection: Does a yellowish whey clarify above a coherent curd?
   - Drain the curd into a sieve/filter cloth, rinse briefly with 50–100 mL of lukewarm water.
   - Press in cloth/mold for 10–20 minutes with 0.5–2 kg (treat all molds identically; record pressure/time).
4. **Weighing and Documentation**
   - Weigh the pressed "tofu/curd" (g).
   - Measure and record the whey pH.
   - Describe the texture (crumbly/firm/glassy/no gel). Take a photo with a scale.
   - Weigh approximately 5 g (=mhyg) of the obtained mass, crumbled, into a weighed porcelain dish (=m0), and dry in an oven overnight until the mass is constant (105°C for 16 h). After cooling, determine the dry mass (=mxer).

➢ Calculate the dry weight and the moisture content of the sample.

Brief observations (expected results)

- Soy milk: Significant coagulation at 1.5–2.5 g $CaCl_2 \cdot 2H_2O$/L; cohesive gel, typical yield of 120–200 g of moist tofu per liter (depending on protein and fat content, pressing time).
- Cow's milk: Formation of a curd/quark-like coagulum, especially with $CaCl_2$; yield lower than soy tofu (highly dependent on fat/water content; without rennet/acid, the curd becomes softer).
- Oat drink: Usually no stable gel formation; possibly fine flakes/cloudiness, very low yield. Consistency may appear slimy due to β-glucans/starches.
- Protein shake: Varying results possible depending on the powder's composition.

Explanation: Protein types and contents vary greatly; soy and casein proteins cross-link effectively with Ca^{2+}, while oats contain little protein and many polysaccharides.

Disposal Instructions

- ✓ Liquid waste (whey/supernatants) without significant chemical additives:
  - When $CaCl_2$/$CaSO_4$ is used in the trace amounts specified here, diluted, uncolored whey can generally be disposed of down the drain; follow local regulations.
- ✓ Residues containing calcium:
  - Collect small amounts of highly saline solutions and dilute them, or dispose of them with inorganic, saline waste (institutional policy).
- ✓ Solid residues (filter cloths, coagulation residues): Dispose of with household waste.
- ✓ Equipment/work surfaces: Clean with warm water and a mild detergent.

Denaturation of Proteins by Various Factors

Objective

To demonstrate and compare the denaturation of proteins under three conditions: pH change, temperature, and the addition of ethanol. The following will be observed: changes in solubility, turbidity, and flocculation (clear/turbid/flocculent), as well as the reversibility of the reaction.

Chemicals and Materials Used

- Protein source(s), depending on availability:
  - Sample from the previous day (filtered/diluted 1:10)
  - Egg white solution from a chicken egg (diluted 1:10 with distilled water)
- pH modulators:
  - Hydrochloric acid (HCl) 1.0 M
  - Sodium hydroxide (NaOH) 1.0 M
- Ethanol 96% (chilled)
- Distilled water

Safety

HCl/NaOH are corrosive; ethanol is highly flammable. Wear PPE; keep ethanol away from ignition sources; work preferably in a fume hood. Water baths reach high temperatures.

Equipment and Materials

- Test tubes/15 mL centrifuge tubes (at least 12–15 per group) or small beakers
- Dropper pipettes; graduated cylinders
- Water bath/hot plate (25–95 °C), thermometer
- Ice (if available)
- Labeling materials

Sample preparations and exact quantities

Prepare a uniform protein base, e.g., 20 mL of egg white solution diluted 1:10 from the previous day and 20 mL of egg white solution diluted 1:10. Volume per test tube: 2.0 mL of each sample solution

1. pH denaturation (pH lowering/raising)

- Target pH values: 3.0; 4.5; 10.0
- Procedure per sample (2.0 mL protein solution):

Note: The exact volumes required depend on the buffer capacity of the protein solution — add drop by drop and swirl well; do not add all at once.

- pH 3.0: 2.0 mL protein + approx. 40–80 µL 1.0 M HCl (~2 drops).
- pH 4.5: 2.0 mL protein + approx. 20–50 µL 1.0 M HCl (~1 drop).
- pH 10.0: 2.0 mL protein + approx. 40–100 µL 1.0 M NaOH (~2 drops).

After homogenization, let stand for 5 min at room temperature and document your observations. Now try to neutralize the three solutions again by adding the same amount of base/acid. Check the pH value and document your observations in the experimental notes.

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Disposal Instructions

- Acid/alkali residues (HCl/NaOH, diluted): Dispose of down the drain after neutralization (pH 6–8) and in accordance with laboratory regulations; observe local regulations.
- Filter out solids beforehand and dispose of them in household waste.

2. Temperature denaturation

- Prepare 4 samples, each containing 2.0 mL of protein solution per test tube (label)
- Conditions: Prepare 4 water baths
    - 20–25 °C (control, 10 min)
    - 45 °C (10 min)
    - 65 °C (10 min)
    - 90 °C (10 min)
- Procedure:

Place test tubes in preheated water baths; start the timer. Remove after the time has elapsed (caution: hot), document observations, and then allow to cool to room temperature. Has anything changed?

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Disposal Instructions

- Dispose of cooled liquid down the drain according to laboratory regulations; observe local rules.
- Filter out solids beforehand and dispose of them in household waste.

3. Ethanol Denaturation (Coagulation by solvent)

- Target final ethanol concentrations: 30%, 50%, 70% (v/v); control 0%
- Ethanol evaporates quickly, so work quickly and in a cool environment or on ice
- Procedure per sample (final volume 2.0 mL):
  - 30% v/v: 1.4 mL protein solution + 0.6 mL 96% ethanol
  - 50% v/v: 1.0 mL protein solution + 1.0 mL 96% ethanol
  - 70% v/v: 0.6 mL protein solution + 1.4 mL 96% ethanol
  - 0%: 2.0 mL protein solution + 0 mL ethanol (control)
  - Mix gently after addition, let stand on ice/at room temperature for 10 min.
- After documenting the observation, add 2 mL of distilled water to reduce the alcohol concentration.

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Disposal Instructions

- Dispose of waste containing ethanol (≥10–15% v/v) with organic solvent waste. Do not pour down the drain; follow local regulations.
- Filter out solids first and dispose of them with household waste.

Observation (expected)

- pH:
  - Significant turbidity/flocculation near the isoelectric point (e.g., pH ~4.5 for many proteins).
  - High/alkaline pH values can also cause denaturation, often with slower-forming visible aggregates.
- Temperature:
  - 65–90 °C: Protein coagulates significantly (white flakes/turbidity/coagulate).
- Ethanol:
  - Increasing ethanol concentration → stronger precipitation; at ≥50–70% v/v, significant flocculation, clear sediment after a short resting period/centrifugation.

Xanthoprotein reaction (nitration of aromatic amino acids)

Detection of tyrosine/tryptophan in dried samples from Day 1 of the experiment

Objective

Detection of aromatic amino acids (primarily tyrosine and tryptophan) in the dried protein samples (dry matter) from the tofu/precipitation experiments using the xanthoprotein reaction with concentrated nitric acid; a color change indicates the presence of aromatic residues.

Chemicals Used

- Concentrated nitric acid HNO_3 (approx. 65–70% w/w) — nitrating reagent
- Sodium hydroxide solution (NaOH) 2 M (for neutralization and color change)
- Distilled water (for dilution/rinsing)
- Dried protein samples (dry weight) from Day 1 of the experiment: soy tofu dry weight, cow's milk dry weight, oats dry weight
- Positive control: small amount of pure tyrosine powder or BSA (for comparison)
- Negative control: cellulose/flour (no aromatic amino acids)

Caution: HNO_3 is highly oxidizing and corrosive.

Equipment and Glassware

- Fume hood (if available)
- Test tubes or small beakers (preferably acid-resistant), pipettes (glass or PTFE),
- Pasteur pipettes, or micropipettes with acid-resistant tips
- Small porcelain dishes (preferably for solid samples) or drip pans
- Spatula/tweezers, labeling materials
- Neutralization container (for small amounts of acid residue) and a sturdy, acid-
- resistant waste container
- pH strips (optional, for testing after NaOH addition)
- Hot plate (if the reaction does not start on its own)

Safety Instructions (Must be read before starting the experiment)

- Work in a fume hood (if available); do not hold your hands over the reagents.
- HNO_3 can release nitrous gases and toxic fumes; therefore, turn on the fume hood fan and ensure proper ventilation.
- Avoid contact with the acid — wear safety goggles, gloves, and a lab coat. In case of skin contact: rinse immediately with plenty of water; seek ophthalmological treatment in case of eye contact.
- Keep organic solvents/corrosive substances away (reduces fire and explosion risk, but organic materials can react violently with HNO_3).
- Use small amounts (drop rule: 1 drop ≈ 50 µL). Do not pour large volumes of concentrated acid.
- Waste: Place acid residues and solids treated with HNO_3 in labeled containers for highly oxidizing/corrosive waste. Do not dispose of down the drain.

Sample preparation (dried samples)

- Weigh the cooled samples from the first day of the experiment to calculate the dry weight and water content (see script "5. Tofu production"). If not yet dried: Dry according to the protocol beforehand and allow to cool in a desiccator.
- Use approx. 5–10 mg of dried dry matter per sample (finely ground).
- Clearly label the small dishes/test tubes (soy, cow, oat, negative control).

Procedure

Note: This procedure uses small amounts of solid material, not solutions, to test the concentration of aromatic compounds in dry matter.

1. Preparation
   - Set up all materials in the fume hood. Wear full PPE.
   - Calculate and prepare 2 M NaOH for subsequent neutralization
2. Positioning the samples
   - For each sample: Place 5–10 mg of dried, finely ground sample on a porcelain dish.
3. Addition of acid (Caution!)
   - Using a Pasteur pipette, very carefully add 1–2 drops (≈ 50–100 µL) of concentrated HNO_3 to the sample.
   - Observe the immediate reaction: a yellow color developing around the sample is typical.
   - If no visible reaction occurs after 30–60 s: gently warm the dish by briefly holding it over a warm hot plate
4. Color intensification / color change (Caution!)
   - After the reaction is complete, carefully add 1–2 drops of 2 M NaOH to convert the yellow nitro color to orange/red (pH-dependent color change).
   - Do not add NaOH directly to excessively large amounts of HNO_3—always add drop by drop and work under a fume hood.
5. Documentation (use the table below)
   - Photograph the samples and note the respective color (yellow → orange → red), intensity (weak/medium/strong), and time to coloration (seconds).
   - Compare with the positive and negative controls.

Disposal (Follow institute guidelines)

- ✓ After the experiment is complete, carefully place any solids contaminated with HNO_3 into the designated waste container. Dilute small residues with water and pour them into the designated acid waste container.
- ✓ Dispose of all used pipettes as chemically contaminated waste.
- ✓ Rinse the work surface with water; remove and dispose of gloves (or clean them if reusable).

Observation — typical results

- Positive (aromatics present, e.g., BSA, dried soy protein mass): immediate yellow coloration, turning intense orange/red upon addition of NaOH.
- Cow's milk dry matter (casein & whey residues): yellow coloration, typically moderate in intensity (depending on the Tyr/Trp ratio).
- Soy dry matter: yellow to strong yellow/orange coloration (soy proteins contain Tyr residues).
- Oat dry matter: weaker or slower coloration (oat proteins tend to have fewer aromatic residues or matrix interferences); however, depending on the sample, a distinct hue may also develop.
- Negative control (cellulose, flour): no discoloration or only very weak discoloration.

Interpretation

- The xanthoprotein reaction is qualitative: color indicates the presence of aromatic amino acids, not the total protein content.
- Color intensity ≠ exact quantity (only a rough estimate is possible).
- Some food ingredients (e.g., aromatic additives, colorants) can distort the results—hence the importance of controls.
- The reaction is irreversible (chemical nitration), so perform this procedure only on samples that cannot be reused.