Tishk International University
Faculty of Applied Science
Nutrition and Dietetics Department
Spring semester
2nd Grade

Nutritional Biochemistry II

Qualitative Determination of Enzymes

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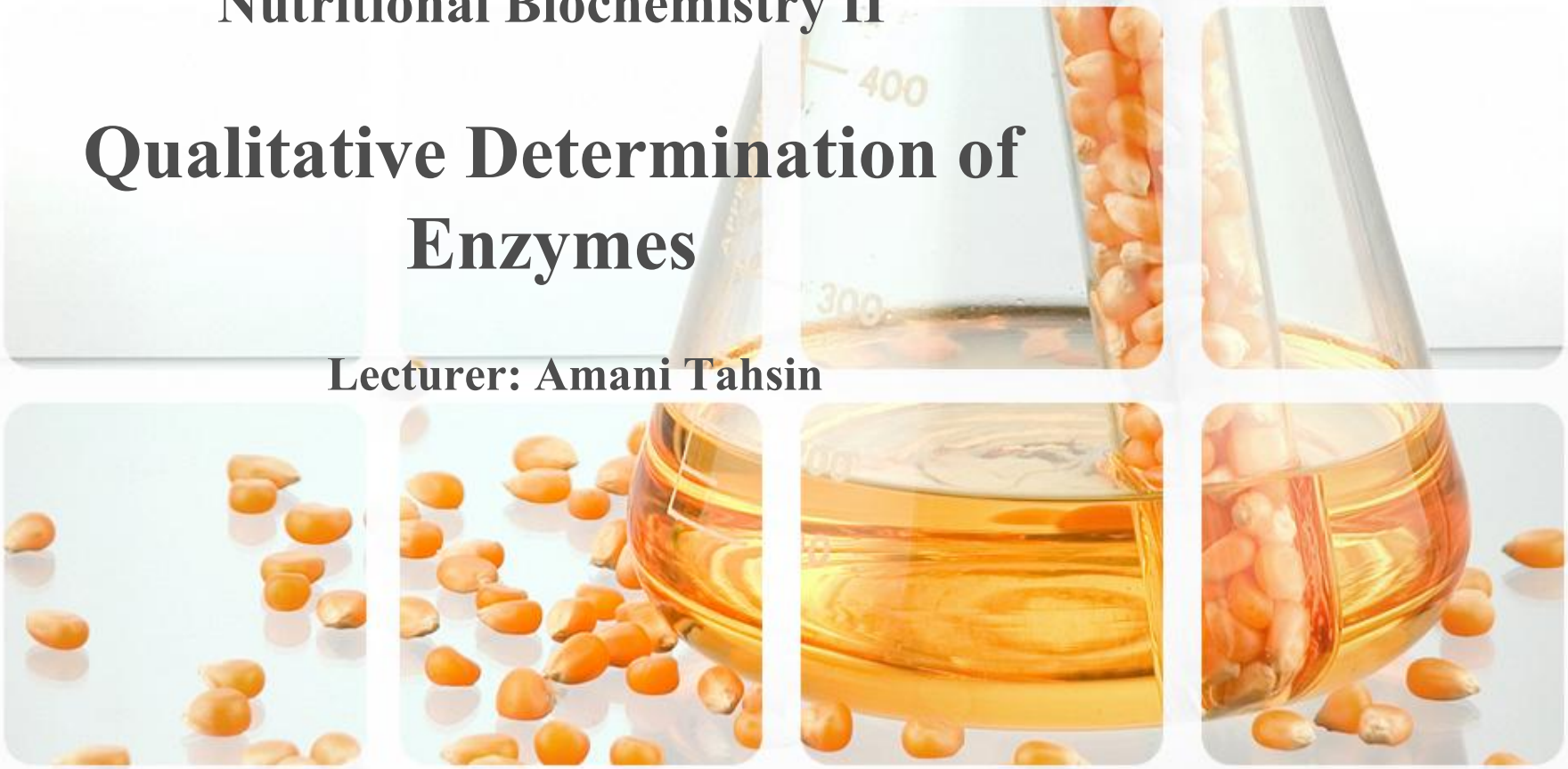

Objectives

- Qualitative enzyme tests provide a simple and effective method for detecting enzyme activity through observable physical or chemical changes.

- These methods are widely used in clinical, microbiological, and biochemical laboratories for preliminary enzyme identification.

Qualitative determination of enzymes

- Refers to identifying the *presence* and *activity* of a specific enzyme in a sample without measuring its exact concentration or activity rate quantitatively.
- In biochemistry practical courses, qualitative tests are commonly based on:

1. Observable **color change**
2. **Precipitate formation**
3. **Gas production**
4. **Substrate disappearance**
5. **Product formation**

General Principle

Enzymes catalyze specific biochemical reactions.

If the enzyme is present and active → it converts a substrate into a product → a visible change occurs.

Common Qualitative Enzyme Tests

1. Catalase Test

Principle:
Catalase breaks down hydrogen peroxide into water and oxygen.

$$2H_2O_2 \rightarrow 2H_2O + O_2\uparrow$$

Procedure:

Add a few drops of hydrogen peroxide (H_2O_2) to the sample.

Observe immediately.

Positive result:
✓ Rapid bubble formation (oxygen gas)

Negative result:
✗ No bubbling

2. Amylase Test (Iodine Test)

Principle:
Amylase hydrolyzes starch into maltose.
Iodine gives a blue-black color with starch.

Procedure:

Incubate starch with enzyme sample.

Add iodine solution.

Positive result (amylase present):
✓ No blue-black color (starch digested)

Negative result:
✗ Blue-black color remains

3. Urease Test

Principle:
Urease breaks down urea into ammonia and CO_2.
Ammonia increases pH.

Procedure:

Add sample to urea broth containing phenol red.

Positive result:
✓ Pink/red color (alkaline)

Negative result:
✗ Yellow/orange (no pH change)

4. Protease Test

Principle:
Protease digests proteins (e.g., casein in milk agar).

Procedure:

Inoculate sample on milk agar.

Incubate.

Positive result:
✓ Clear zone around colony (protein hydrolysis)

Negative result:
✗ No clearing

5. Lipase Test

Principle:
Lipase hydrolyzes fats into fatty acids and glycerol.

Observation methods:

Formation of clear zones on tributyrin agar

Color change using pH indicators

Important Factors Affecting Qualitative Tests

- Temperature
- pH
- Incubation time
- Enzyme concentration
- Substrate specificity

Precautions

- Maintain optimal temperature (37°C unless stated otherwise).
- Use fresh reagents.
- Avoid contamination of samples.
- Handle hydrogen peroxide carefully.
- Record observations immediately.