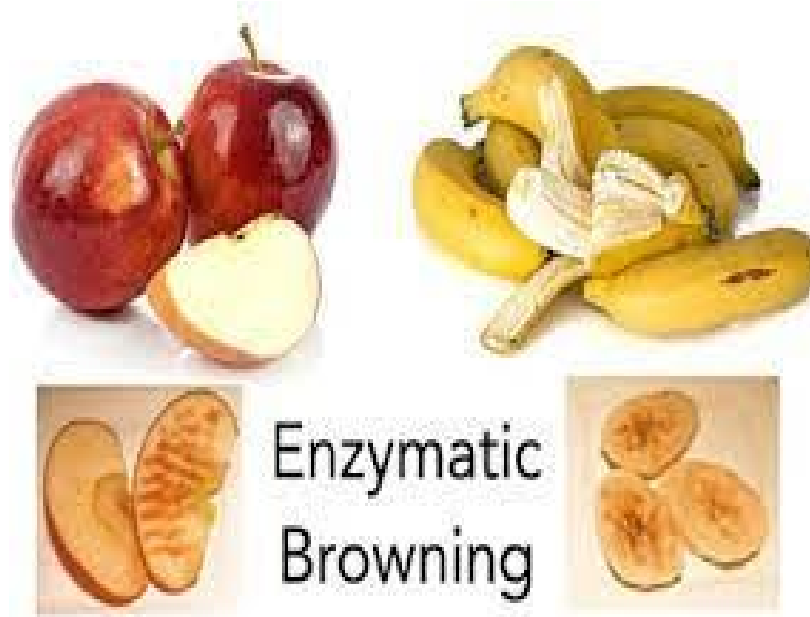


# Practical Biotechnology

## Lab 9

### Extraction of tyrosinase

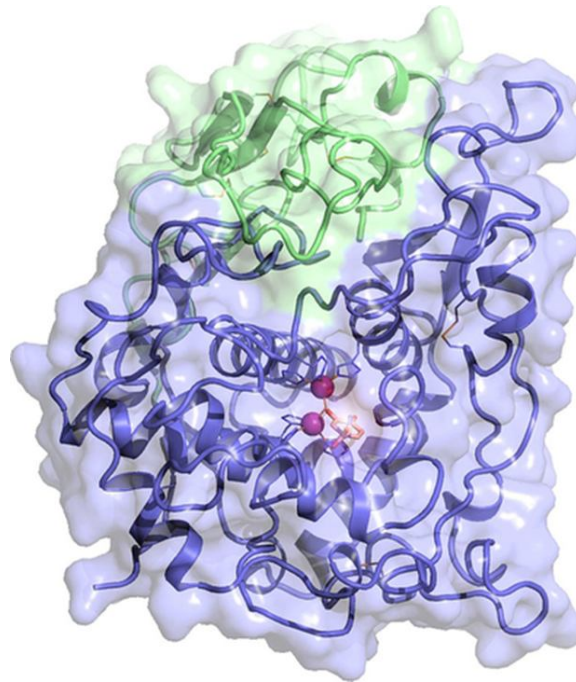
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# Tyrosinase

Tyrosinase is an enzyme that catalyzes dihydroxyphenylalanine (DOPA) in the pathway of melanin synthesis And plays an important role in the melanogenesis and enzymatic browning.


Therefore, its inhibitors can be attractive in cosmetics and medicinal industries as depigmentation agents.



**Tyrosinase** is a key enzyme in the biosynthesis of melanin ,involved in determining the color of skin, eyes and hair in mammals.

Studies of tyrosinases were motivated by the need to understand and prevent the enzymatic browning that occurs in the presence of air when mushrooms, fruits or vegetables are cut or bruised.

This phenomenon is related to the tyrosinase activity and causes severe economic losses in the food industry.



**Why do fruits and vegetables turn Brown??**

Oc1ccc(O)cc1 (2,4-dihydroxyphenyl)  $\xrightarrow[\text{Copper Polyphenol Oxidase}]{\text{Oxygen}}$  O=C1C=CC(=O)C=C1 (o-quinone)  $\rightarrow$  Melanin (Browns Pigmentation)

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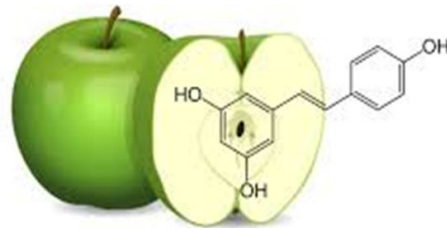
When cells have been damaged by the inflammation of a pimple, the Tyrosinase enzyme will send an excess of Tyrosine which will result in overproduction of pigment. This is what you see as a scar.

Research has established the best way to control an excess of melanin production is to target this crucial enzyme.



## sources

- Tyrosinases have been isolated and purified from different sources such as some plants, animals and microorganisms.
- Recently, a novel tyrosinase produced by Sahara soil actinobacteria have been isolated.
- However, among different sources of tyrosinase, mushroom tyrosinase from *Agaricus bisporus* is a major and cheap source of tyrosinase with high similarity and homology compared to human tyrosinase.





# Applications

## In the Cosmetic Industry

Cosmetic companies have been focused on developing novel whitening agents that selectively suppress the activity of tyrosinase to reduce hyperpigmentation .

## In the food industry

highly effective tyrosinase inhibitors are needed in agriculture and the food industry. Well known tyrosinase inhibitors include vanillic acid, vanillin, and vanillic alcohol.

## Procedure

1. Peel a small potato and cut into pieces approximately 1-inch square.
2. Add 100 grams of the potato to a blender, along with 100 mL of sodium fluoride (NaF). Homogenize for about 1 minute at high speed.

**Caution:** Sodium fluoride is a poison! Wear rubber gloves while handling, and wipe up any spills immediately.

3. Pour the homogenate (mixture) through several layers of cheesecloth into a beaker.
4. Measure the volume of the homogenate and add an equal volume of saturated ammonium sulfate.

(That is, if the fluid volume of your homogenate is 150 mL, add 150 mL of ammonium sulfate. This will cause a flocculent white precipitate to appear, as many of the previously soluble potato proteins become insoluble. The enzyme tyrosinase is one of these proteins, and thus will be found in the subsequent precipitate).

5. Divide the ammonium sulfate-treated homogenate into chilled centrifuge tubes and centrifuge at 1500 xg for 5 minutes at 4°C.
6. Collect the centrifuge tubes, and carefully pour off and discard the fluid (supernatant). Save the pellets. Combine all of the pellets into a 100-mL beaker.
7. Add 60 mL of citrate buffer, pH 4.8, to the pooled pellet and stir the contents well. Use a glass rod to break up the pellet. Continue to stir for 2 minutes while keeping the solution cool.



8. Again divide the solution into centrifuge tubes and recentrifuge at 300 xg for 5 minutes at 4°C.

9. Collect and save the supernatant. This is your enzyme extract! Place it in an erlenmeyer flask, label it as “enzyme extract” and place it in an ice bucket.

The enzyme tyrosinase is insoluble in 50% ammonium sulfate, but is soluble in the citrate buffer. Keep this extract chilled for the duration of the experiment.



Thank you for  
listening

