بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

رَبِّ اشْرَحْ لِیْ صَدْرِیْ O وَیَسِّرْلِیْ اَمْرِیْ O
وَ ا حْلُلْ عُقْدَةً مِّنْ لِّسَانِیْ O یَفْقَهُوْا قَوْلِیْ O

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میری زبان کی گرہ کھول دے تا کہ لوگ میری بات سمجھ سکیں

رَّبِّ زِدْنِی عِلْمًا

My Lord! Increase me in knowledge.

FST-311. L # 28:
ENZYMATIC BROWNING

ENZYMATIC BROWNING

Definition

- A **chemical** process which occurs in **Fruits** and **Vegetables** by the enzyme **Polyphenol oxidase** (**Catechol oxidase**, others), which results in **Brown** pigments (**Melanin, Benzoquinone**)
- **Oxidation of Foods**

Occurrence

- Fruits (**Apple**, Apricots, Pears, Bananas, Grapes)
- Vegetables (**Potatoes**, Mushrooms, Lettuce)
- Seafood (**Shrimps**, Spiny lobsters, Crabs)

DISADVANTAGE OF ENZYMATIC BROWNING

Food Quality Loss

- Enzymatic Browning is detrimental to **Quality**, particularly in **Post-Harvest** & **Storage** of Fresh fruits, vegetable, Juices and some Shellfish
- PHL: **30-40** %
- Enzymatic Browning may be responsible for up to **50 %** of all **Losses** during fruit and vegetables **Storage, Processing** & **Production**

ENZYMATIC BROWNING - ESSENTIALITY

On the other hand Enzymatic Browning is essential for the **Color** and **Taste** of

- **Tea**
- **Coffee**
- **Chocolate**
- Developing color and flavor in Coffee, Cocoa Beans, and Tea
- Developing color and flavor in dried fruit such as Figs and Raisins

POLYPHENOLS – MAIN COMPONENTS IN EB

Polyphenols

- Polyphenols, also called phenolic compounds, are group of chemical substances present in plants (**fruits** & **vegetables**) which play an important role during enzymatic browning, because they are **substrates** for the **browning-enzymes**

ORIGINAL "WBSSH" DEFINITION OF POLYPHENOLS

The WBSSH describes the polyphenol class as:

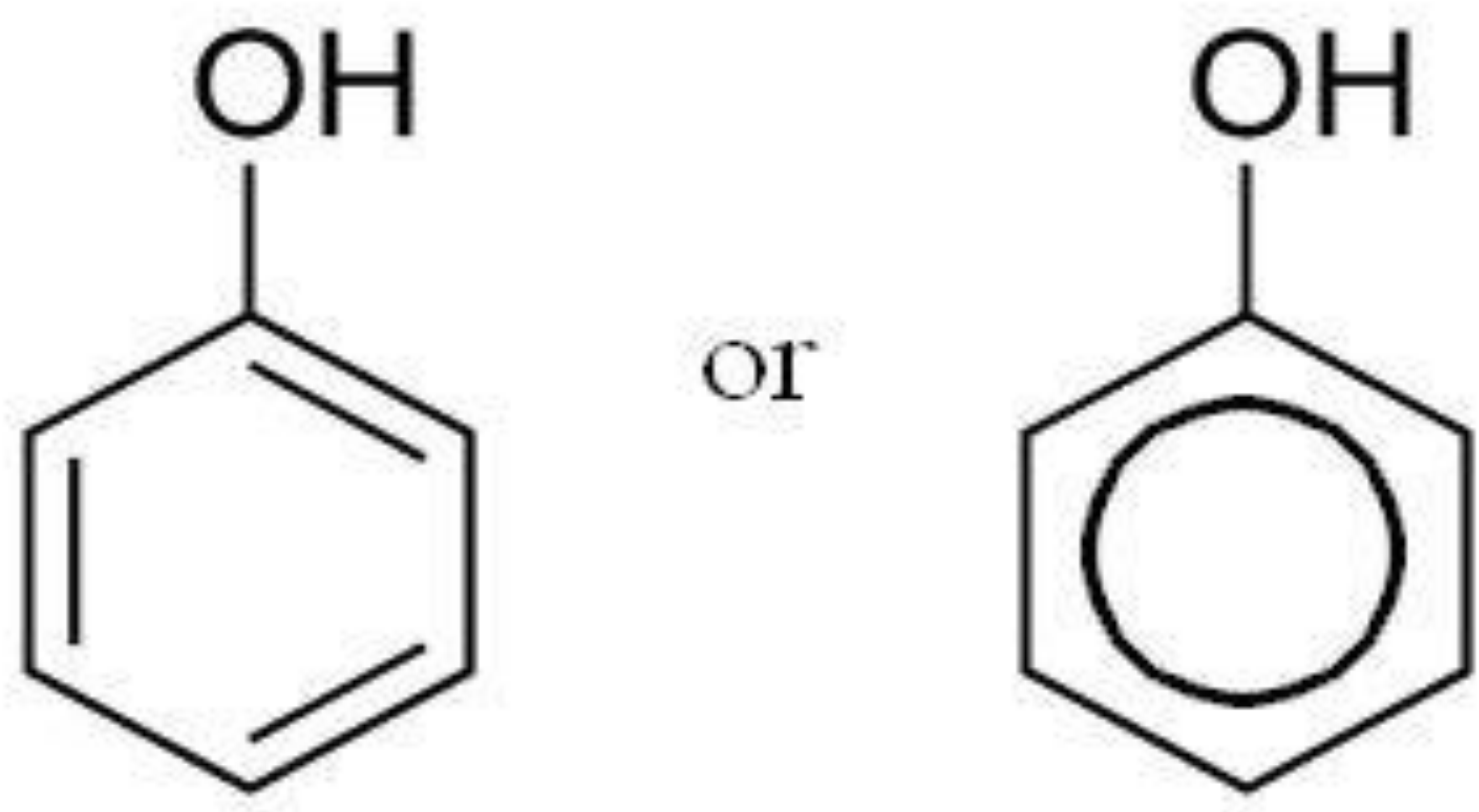
- Generally moderately water-soluble compounds
- with molecular weight of **500–4000 Da**
- With **>12 phenolic hydroxyl** groups
- With **5–7 aromatic rings** per 1000 Da

where the limits to these ranges are somewhat flexible

(White–Bate-Smith–Swain–Haslam)

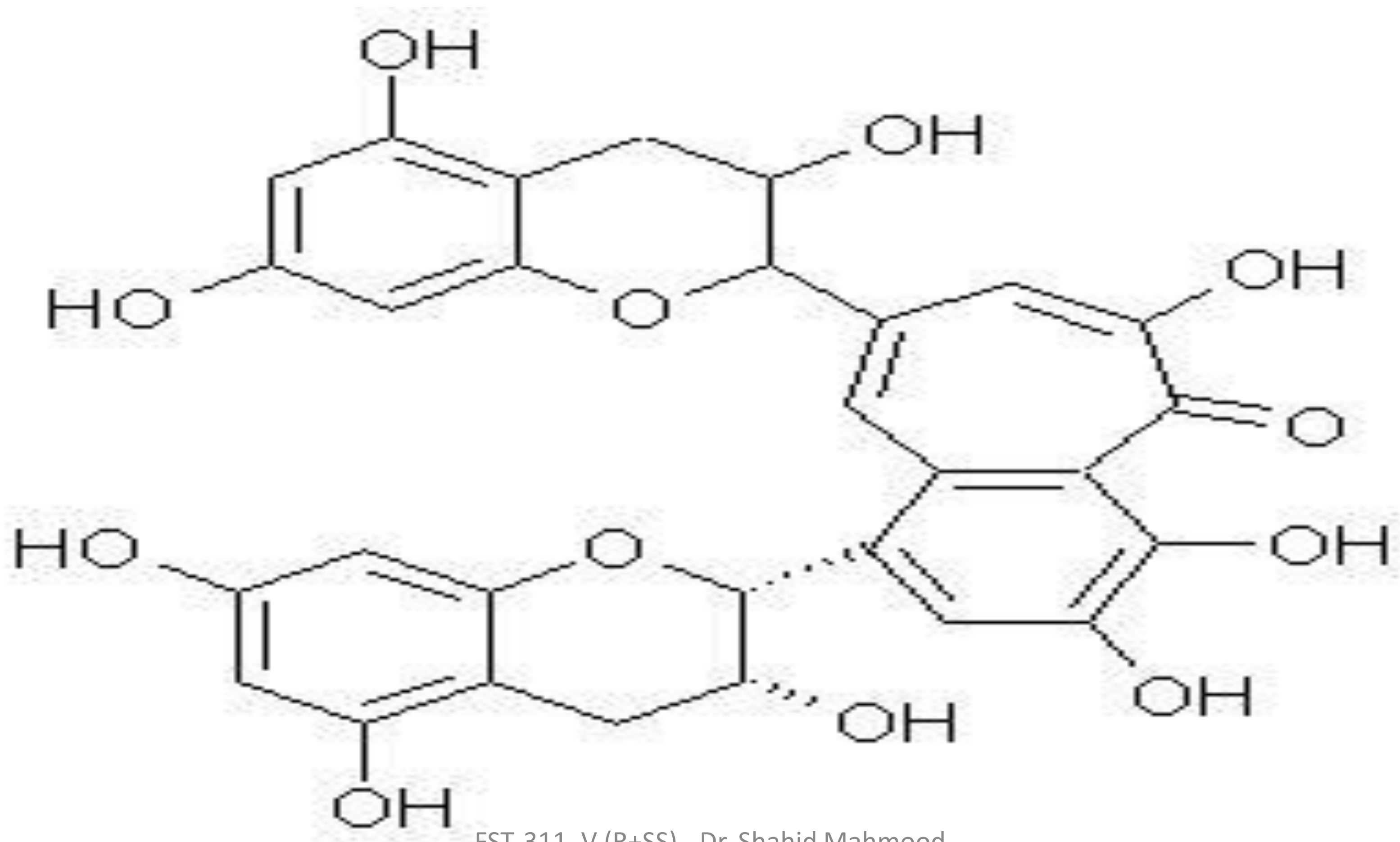
POLYPHENOLS – MAIN COMPONENTS IN EB

Phenol



POLYPHENOLS – MAIN COMPONENTS IN EB

Theaflavin (polyphenol) in Tea



POLYPHENOLS – MAIN COMPONENTS IN EB

Natural Role in Plants

- Phenolic compounds are **responsible** for the **color** of many plants, such as **Apples**
- Part of the **Taste** and **Flavor** of beverages (**Apple juice, Tea**)
- Important **Anti-oxidants** in plants
- Polyphenols are normally complex organic substances, which contain more than one **Phenol group** (**Carbolic acid**)

POLYPHENOLS – MAIN COMPONENTS IN EB

Polyphenols Sub-classes

- Anthocyans (Color in **fruits**)
- Flavonoids (**Catechins**, **Tannins** in **Tea** and **Wine)**
- Non-flavonoids components (**Gallic acid** in Tea leaves)

Flavonoids Formation

- Flavonoids are formed in plants from the **Aromatic amino acids**
  - **Phenylalanine**
  - **Tyrosine**

POLYPHENOLS – MAIN COMPONENTS IN EB

FOOD PROCESSING AND STORAGE

- During **Food Processing** and **Storage** many **Polyphenols** are **UNSTABLE** due to the fact that they undergo **Chemical** and **Biochemical** reactions
- The most important is **Enzymatic Oxidation** causing **Browning** of **Vegetables** & **Fruits**
- This reaction mostly occurs after **Cutting** or other **Mechanical injuries / treatments** of product due to **Breaking Cells**

CITATION

- Quideau, S. P.; Deffieux, D.; Douat-Casassus, C. L.; Pouységu, L. (2011). "Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis". Angewandte Chemie International Edition. 50 (3): 586–621. Bibcode:2012AnChe..51.3695M. doi:10.1002/anie.201000044. PMID 21226137.
- Haslam, E.; Cai, Y. (1994). "Plant polyphenols (vegetable tannins): Gallic acid metabolism". Natural Product Reports. 11 (1): 41–66. doi:10.1039/NP9941100041. PMID 15206456

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رَبِّ زِدْنِی عِلْمًا

MY LORD! INCREASE ME IN KNOWLEDGE.

FST-311. L # 29: SUBSTRATES/POLYPHENOLS; INVOLVED IN ENZYMATIC BROWNING

SUBSTRATES/POLYPHENOLS; INVOLVED IN EB

FOODS	SUBSTRATES / PHENOLIC SUBSTRATES
Apple	Chlorogenic acid (flesh), Catechol, Catechin (peel), Caffeic acid, 3,4-Dihydroxyphenylalanine (DOPA), 3,4-Dihydroxy benzoic acid, p-Cresol, 4-Methyl catechol, Leucocyanidin, p-Coumaric acid, Flavonol glycosides
Apricot	Isochlorogenic acid, Caffeic acid, 4-Methyl catechol, Chlorogenic acid, Catechin, Epicatechin, Pyrogallol, Catechol, Flavonols, p-Coumaric acid derivatives
Avocado	4-Methyl catechol, Dopamine, Pyrogallol, Catechol, Chlorogenic acid, Caffeic acid, DOPA

SUBSTRATES/POLYPHENOLS; INVOLVED IN EB

FOODS	SUBSTARTES / PHENOLIC SUBSTRATES
Banana	3,4-Dihydroxyphenylethylamine (Dopamine), Leucodelphinidin, Leucocyanidin
Cacao	Catechins, Leucoanthocyanidins, Anthocyanins, Complex Tannins
Coffee Beans	Chlorogenic acid, Caffeic acid

SUBSTRATES/POLYPHENOLS; INVOLVED IN EB

FOODS	SUBSTRATES / PHENOLIC SUBSTRATES
Eggplant	Chlorogenic acid, Caffeic acid, Coumaric acid, cinnamic acid derivatives
Grape	Catechin, Chlorogenic acid, Catechol, Caffeic acid, DOPA, Tannins, Flavonols, Protocatechuic acid, Resorcinol, Hydroquinone, Phenol
Lettuce	Tyrosine, Caffeic acid, Chlorogenic acid derivatives
Lobster	Tyrosine
Mango	Dopamine-HCl, 4-Methyl catechol, Caffeic acid, Catechol, Catechin, Chlorogenic acid, Tyrosine, DOPA, *P*-Cresol

SUBSTRATES/POLYPHENOLS; INVOLVED IN EB

FOODS	SUBSTRATES / PHENOLIC SUBSTRATES
Mushroom	Tyrosine, Catechol, DOPA, Dopamine, Adrenaline, Noradrenaline
Peach	Chlorogenic acid, Pyrogallol, 4-Methyl catechol, Catechol, Caffeic acid, Gallic acid, Catechin, Dopamine
Pear	Chlorogenic acid, Catechol, Catechin, Caffeic acid, DOPA, 3,4-Dihydroxy benzoic acid, *p*-Cresol
Plum	Chlorogenic acid, Catechin, Caffeic acid, Catechol, DOPA
Potato	Chlorogenic acid, Caffeic acid, Catechol, DOPA, *p*-Cresol, *p*-Hydroxyphenyl propionic acid, *p*-Hydroxyphenyl pyruvic acid, *m*-Cresol

SUBSTRATES/POLYPHENOLS; INVOLVED IN EB

FOODS	SUBSTRATES / PHENOLIC SUBSTRATES
Shrimp	Tyrosine
Sweet potato	Chlorogenic acid, Caffeic acid, Caffeylamide
Tea	Flavanols, Catechins, Tannins, Cinnamic acid derivatives

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

PHENOLASES

- **PHENOLASES** which are enzymes found **OUTSIDE** the **CELL WALL** come in contact with **COLORLESS PHENOLS** which are **FOUND INSIDE** the **CELL** causing the brown color to appear
- **PPO** contains **4 Cu^{++}** in 1 molecule and binding sites

DISCOVERY

- **PPO** are a class of enzymes that were first discovered in **MUSHROOMS** and are widely distributed in nature

PRESENCE

- **COLORLESS PHENOLS** to **RESIDE** in the **PLASTIDS** and **CHLOROPLASTS** of plants, although freely existing in the **CYTOPLASM** of **SENESCING** or **RIPENING** plants

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

ROLE IN PLANTS & ANIMAL

- **PPO** is thought to play an important role in the **RESISTANCE** of plants to **MICROBIAL** and **VIRAL** infections and to **ADVERSE CLIMATIC CONDITIONS**
- **PPO** also occurs in animals and is thought to **INCREASE DISEASE RESISTANCE** in **INSECTS** and **CRUSTACEANS**

RESISTANCE DEVELOPMENT ?

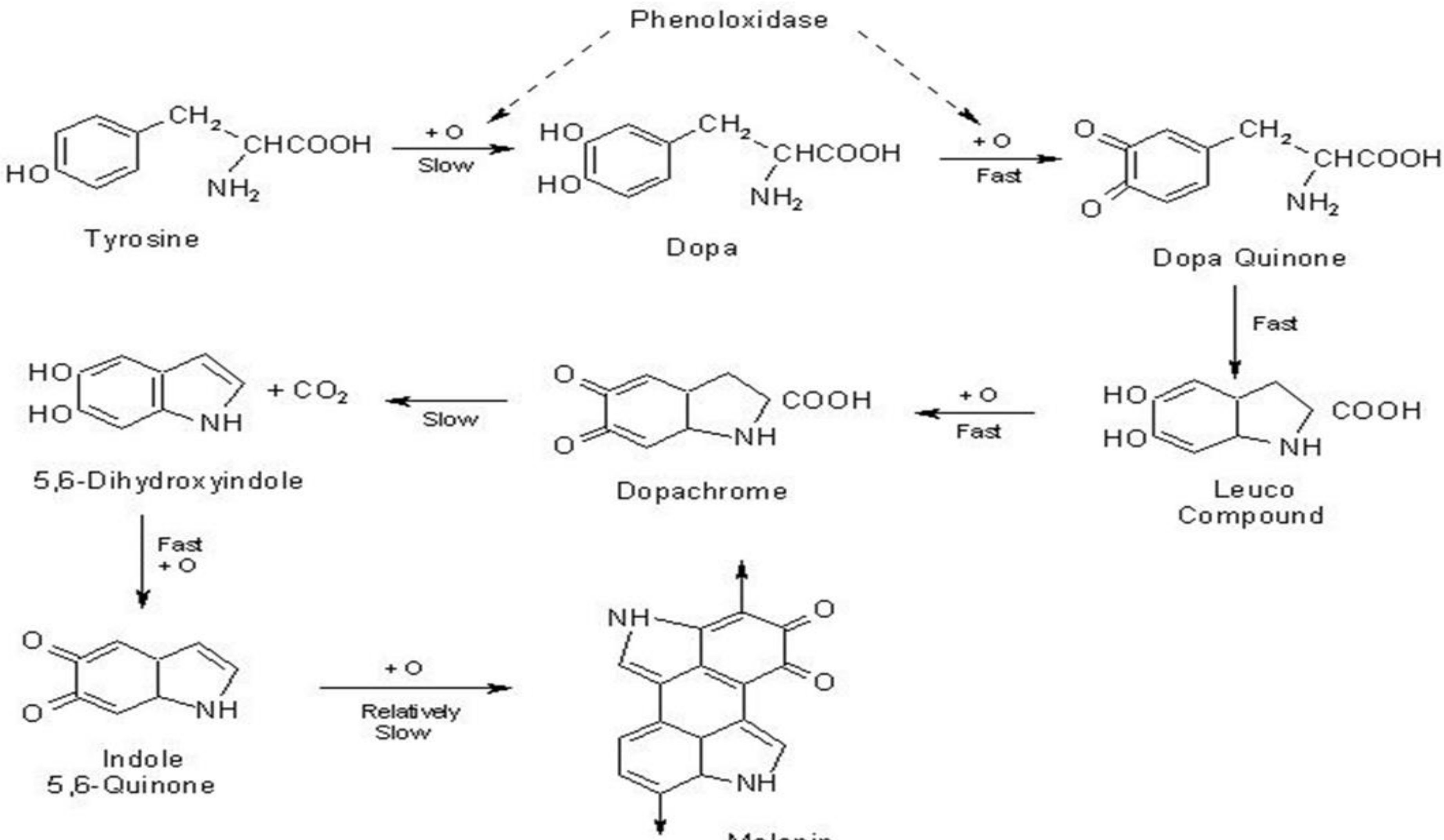
- In the presence of O_2 from air, the enzyme catalyses the first steps in the biochemical conversion of **PHENOLICS** to **PRODUCE QUINONES**, which undergo further **POLYMERIZATION** to yield **DARK**, **INSOLUBLE POLYMERS** referred to as **MELANINS**
- These **MELANINS** form **BARRIERS** and have **ANTIMICROBIAL** properties which prevent the spread of **INFECTION** or **BRUISING** in plant tissues

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

RESISTANCE DEVELOPMENT ?

- Plants, which exhibit comparably **HIGH RESISTANCE TO CLIMATIC STRESS**, have been shown to possess relatively **HIGHER PPO** levels than susceptible varieties
- **PPO** catalyses two basic reactions: **HYDROXYLATION** and **OXIDATION**
- Both reactions utilize molecular $\mathbf{O_2}$ (air) as a **CO-SUBSTRATE**
- The reaction is not only dependent on the presence of air, but also on the **pH** (acidity)
- The **reaction does not occur at acid (pH <5) or alkaline (pH >8)** conditions

FORMATION OF MELANINS FROM A SIMPLE POLYPHENOL (TYROSINE)



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رَّبِّ زِدْنِي عِلْمًا

MY LORD! INCREASE ME IN KNOWLEDGE.

FST-311. L # 30:
PREVENTION OF ENZYMATIC BROWNING-I

PREVENTION OF ENZYMATIC BROWNING

- The control of **BROWNING** is one of **THE MOST IMPORTANT ISSUES** in the **FOOD INDUSTRY**, as **COLOR** is a significant attribute of food which **INFLUENCES CONSUMER DECISION** and brown foods (especially fruits) are seen as **SPOILED**
- Several methods can be applied to **AVOID EB**
  - *By inactivating the enzyme (**HEAT**)* ⇐ **BLANCHING**
  - *By removing essential components (most often **O_2**) from the product*

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

BLANCHING

- **BLANCHING** is a **SHORT HEAT TREATMENT** to **DESTROY** or **INACTIVATE** enzymes **BEFORE FREEZING** of products (mainly vegetables)
- **ENZYME ACTIVITY** may **DISCOLOR** or **TOUGHEN** vegetables during **FREEZING**, which results in **QUALITY LOSS**
- Blanching **BRIGHTENS** the **COLOR**, **SOFTENS** the **TEXTURE**, but has **LITTLE EFFECT** on **NUTRIENT** content or **FLAVOR** as it is a relatively short process
- The blanching **TEMPERATURE** depends on the **TYPE OF ENZYME** which occurs in the product, but is generally between **70-100°C**, sometimes higher when more resistant enzymes are to be inactivated

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

INACTIVATION TEMPERATURE FOR SOME ENZYMES		
Enzymes	**Effects**	**Temp (°C)**
Lipolityc Acyl Hydrolase	Rancidity	≈ 75
Lipoxygenase	Rancidity	≈ 80
Polyphenol Oxidase	Browning	≈ 100
Peroxidase	Deterioration	≈ 135

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

TYPES OF BLANCHING

Blanching in Steam/Boiling Water

- A type of **HEAT** treatment for **CONTROLLING EB** in **CANNED** or **FROZEN** **fruits** and **vegetables**
- It is **SCALDING** the vegetables or food in water or steam for a **SHORT PERIOD OF TIME**
- The **STEAM** blanching is **1.5 times** longer than **BOILING WATER** blanching

Microwave Blanching

- Microwave blanching may **NOT BE EFFECTIVE**, since research shows that **SOME ENZYMES MAY NOT** be inactivated
- This could result in **OFF-FLAVORS** and **LOSS OF TEXTURE** and **COLOR**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

REFRIGERATION

- **REFRIGERATION** and **CHILLING** are used to prevent **SPOILAGE** of **VEGETABLES** and **FRUITS** during **DISTRIBUTION** and **RETAILING**
- **CHILLING** is applied often for **BROCCOLI, BERRIES, SPINACH, PEAS, BANANAS, MANGOES, AVOCADOS, TOMATOES**
- At temperatures **BELOW 7 °C** the **PPO** enzyme activity is **INHIBITED**, but the **ENZYME IS NOT INACTIVATED**. Therefore the temperature should be well controlled

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رَّبِّ زِدْنِي عِلْمًا

My Lord! Increase me in knowledge.

FST-311. L # 31:
PREVENTION OF ENZYMATIC BROWNING-II

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

FREEZING

- Like **REFRIGERATION**, **FREEZING INHIBITS**, **BUT NOT INACTIVATES** the **enzyme**
- After **THAWING**, the enzyme **ACTIVITY will RESUME**

CHANGE pH

- The enzyme activity is **pH DEPENDENT**
- Lowering of the **pH to 4.0** by the addition of **CITRIC**, **ASCORBIC** or other **ACIDS INHIBITS** the **ENZYME ACTIVITY**
- During home-preparation of vegetables or fruits **LEMON JUICE** or **VINEGAR** is often sprinkled on the fruit to prevent **BROWNING**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

DEHYDRATION

- The **REMOVING WATER** molecules from the product
- The **PPO** enzyme **NEEDS SUFFICIENT WATER TO BE ACTIVE**
- By **DRYING** the enzyme is **INHIBITED, BUT NOT DESTROYED**
- To **avoid** **FLAVOR** and **QUALITY LOSS**, dehydration should **NOT INVOLVE HEAT**
- **SUN DRYING IS HISTORIC**

COMMON METHODS FOR DEHYDRATION ARE

- **FREEZING-DRYING** when moisture is removed by **SUBLIMATION** (the change from solid to gas); products are **FROZEN** and slowly **DEHYDRATED UNDER VACUUM**
- **LOWERING WATER ACTIVITY** by adding **WATER-BINDING CHEMICALS** and the most commonly used substances are salt (**Sodium chloride**), **Sucrose**, and other **Sugars**, **Glycerol**, **Propylene Glycol** and **Syrups** or **Honey**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

IRRADIATION

- **IRRADIATION**, or as it is sometimes called "**COLD PASTEURIZATION**", is a process in which food is submitted to **IONIZED RADIATION** in order to **KILL BACTERIA** and **REDUCE** the **ENZYME ACTIVITY**
- Irradiation is often applied in **MEATS, SEAFOOD, FRUITS, VEGETABLES and CEREAL** grains for long-term preservation
- Several types of irradiation methods are used in food processing
  - *Gamma Rays*
  - *X-Rays*
  - *Accelerated Electrons (electron beams)*
- **Disadvantages** of radiation are **LOSS OF NUTRIENTS** and **LOW CONSUMER ACCEPTANCE**
- **IRRADIATION IS THUS RARELY USED**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

HIGH PRESSURE TREATMENT

- **HIGH PRESSURE TREATMENT/HIGH PRESSURE PROCESSING (HPP)** is a technique of **FOOD PROCESSING** where food is subjected to elevated pressures (**500-700** atm) to **ACHIEVE MICROBIAL** and **ENZYME INACTIVATION**
- High pressure processing causes **MINIMAL CHANGES** in foods
- Compared to **THERMAL PROCESSING, HPP** results in foods with **FRESHER TASTE**, and **BETTER APPEARANCE, TEXTURE** and **Nutrition**
- High pressure processing without heat eliminates thermally induced cooked **OFF-FLAVORS**
- The technology is especially beneficial for heat-sensitive products, but **STILL VERY EXPENSIVE**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

SUPERCRITICAL CARBON DIOXIDE (SC-CO_2)

- **SC-CO_2** (liquid **CO_2** at **High Pressure**) treatment is mostly applied to DESTROYING **MICRO-ORGANISMS** but can also be applied for **ENZYME INACTIVATION**, especially for inactivation of **PPO** in **SHRIMPS**, **LOBSTERS** and **POTATOES**
- **INACTIVATION OF THE ENZYME** is a result of a **DECREASE IN pH** caused by production of **CARBONIC ACID** from **CARBON DIOXIDE**

ADDITION OF INHIBITORS

- Inhibitions can act in three ways
  - ***Inactivation of enzyme*** *(acting directly on the enzyme)*
  - ***Inactivation of substrate*** *(removing the substrate like O_2 or Phenolics)*
  - ***Inactivation of product*** *(changing the product composition)*
- Large amount of **INHIBITORS** are applied in food processing depending on the **TYPE** of **PRODUCT** and **PROCESS**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

INHIBITORS OF ENZYMATIC BROWNING		
Category	**Inhibitor**	**Mode of Action**
Reducing Agents (Reduction)	**Sulphiting agents Ascorbic acid Analogs Cysteine Glutathione**	**Removal of O_2**
Chelating Agents (Chelation)	**Phosphates EDTA Organic acids**	**Removal of Metals PPO Cu^{++}**
Acidulants (Acidulation)	**Citric acid Phosphoric acid**	**Reducing pH**
Enzyme Inhibitors	**Aromatic Carboxylic acids Peptides Substituted Resorcinols**	**React with Enzymes**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

Ascorbic Acid acts as Antioxidant

- O_2 preferentially oxidized the **ASCORBATE** and not the **PHENOLIC** compounds

Citric Acid acts as a Chelating agent

- Complexes **Cu^{++}** that are necessary for enzyme activity

Acetic Acid a Strong Organic acid

- Reduces the **pH** below **3.0** and irreversibly inactivates the enzyme

H_2O as O_2 Restrictor

- Immersion in **H_2O** restricts the available O_2

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

ULTRAFILTRATION

- Ultrafiltration is a **MEMBRANE SEPARATION** process, driven by a **PRESSURE GRADIENT**
- The membrane **SEPARATES LIQUID** components according to their **SIZE** and **STRUCTURE**
- In the FOOD INDUSTRY this technique is for example applied for **WHITE WINE** and **FRUIT JUICES**
- Ultrafiltration is able to remove **LARGER MOLECULES** like **PPO**, **but not LOWER MOLECULAR** weight components like **POLYPHENOLS**

POLYPHENOL OXIDASE (PPO, PHENOLASES) & EB

ULTRASONICATION

- Ultrasonication is an advanced method to **INACTIVATE ENZYMES**
- Ultrasonic **SOUND WAVES** are able to destroy large molecules by liberating highly reactive **RADICALS** from water
- **It is not yet applied on a large scale**