Enzymatic Browning

Asst. Prof. Cansu Ekin GUMUS

ENZYMATIC BROWNING

Mechanical or physiological damages that may occur during post-harvest storage or processing of fruits and vegetables are among the most important causes of quality loss.

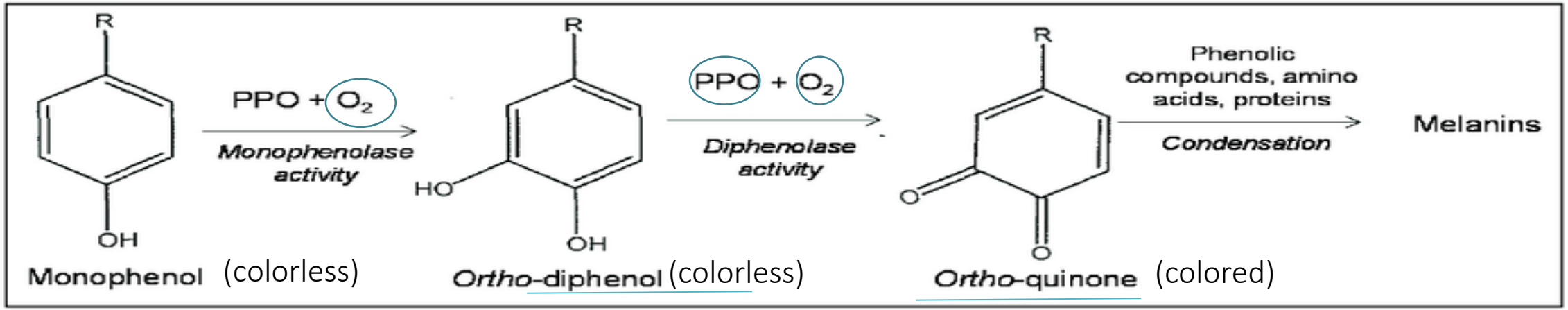
Most of the losses that can occur especially in tropical and subtropical fruits and vegetables are **enzymatic** in origin.

Among the fruits and vegetables where *enzymatic browning (EB)* is a major problem are: Bananas, apples, peaches, pears, avocados, grapes, olives, lettuce, potatoes and mushrooms.

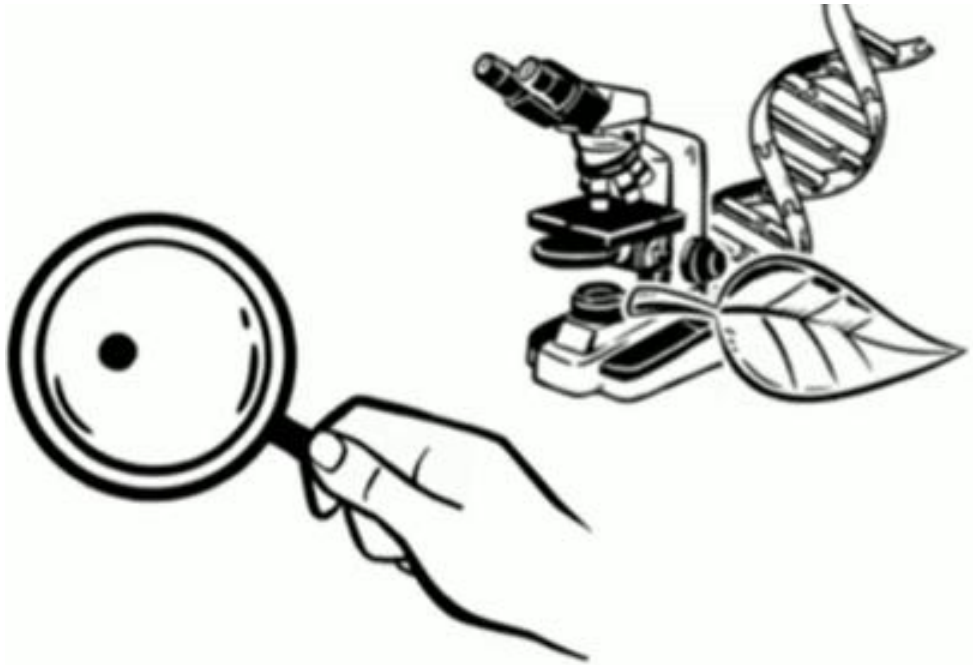
The most important products that EB comes across as a result of the process are: canned fruit slices and fruit juices.

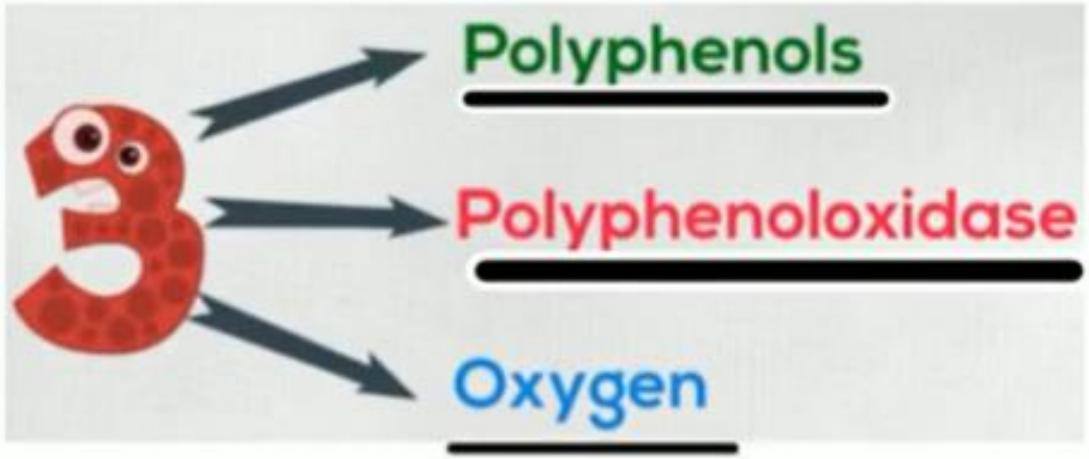
The principal enzyme that plays a role in EB is **Polyphenoloxidase** –PPO. Polyphenol oxidases (PPOs) comprise tyrosinases (TYRs) and catechol oxidases (COs)

EB occurs with the conversion of phenolic compounds to quinones as a result of oxidation in the presence of oxygen under the effect of this enzyme.



These quinones are very reactive compounds and react with other quinones, amino acids or proteins to form dark pigments with high molecular weight. These pigments are called **Melanins** and **Melanoproteins**.




3
Polyphenols
Polyphenoloxidase
Oxygen

Polyphenol oxidase- PPO

PPO enzymes are copper-containing enzymes in their structure and are very common in animals, plants, molds and bacteria.

PPOs were first discovered in mushrooms in 1856.

PPO is very common in nature. It is found in plastids and chloroplasts in plants. It is also found free in ripe fruits. The resulting compounds with the effect of PPO form an antimicrobial barrier against infection and decay.

It has been determined that plants resistant to climate change have high PPO levels.

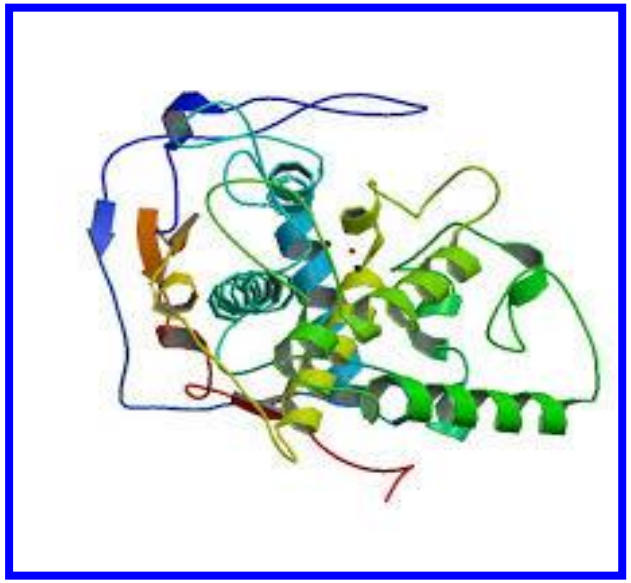
PPO is found in the form of zymogen or pro-PPO in insects and shellfish and provides resistance to diseases in these creatures.

(A zymogen, also called a proenzyme, is an inactive precursor of an enzyme)

According to the substrate specificity, different numbers have been given to oxidases by EC

The Enzyme Commission **number** (**EC number**) by

The **International Union of Biochemistry and Molecular Biology** (IUBMB)

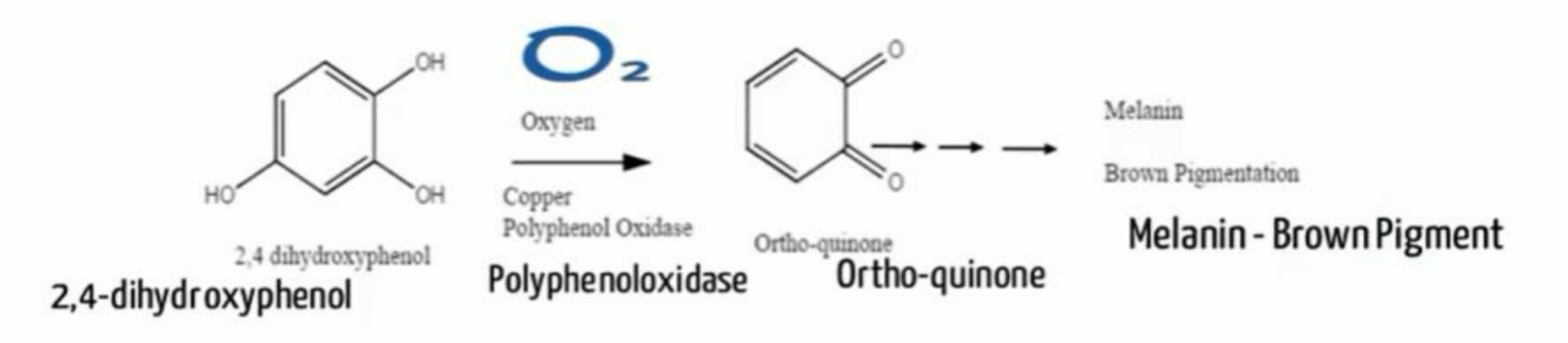


Enzyme Nomenclature: EC 1.10.3.1

Accepted name: catechol oxidase

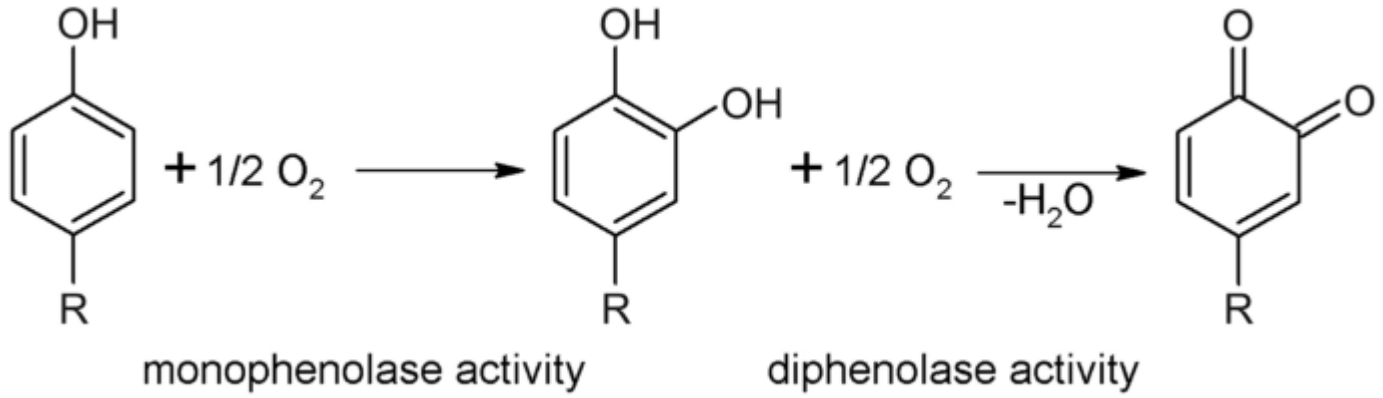
Other name(s): diphenol oxidase; *o*-diphenolase; phenolase; polyphenol oxidase; tyrosinase; pyrocatechol oxidase; Dopa oxidase; catecholase; *o*-diphenol:oxygen oxidoreductase; *o*-diphenol oxidoreductase

Comments: A type 3 copper protein that catalyses exclusively the oxidation of catechols (i.e., *o*-diphenols) to the corresponding *o*-quinones. The enzyme also acts on a variety of substituted catechols.



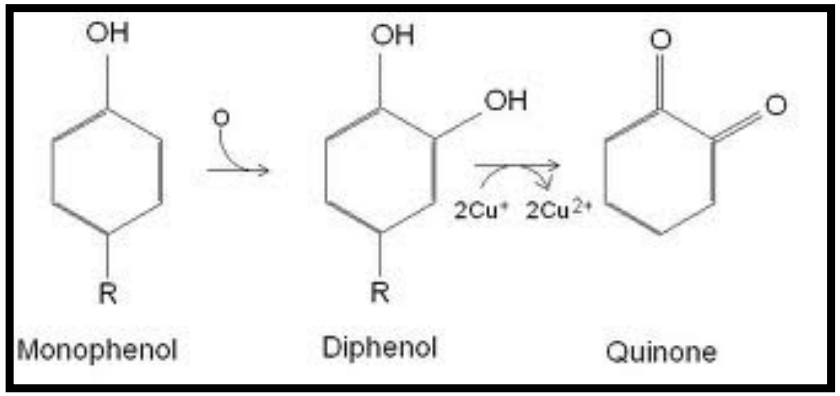
Enzyme Nomenclature: EC.1. 14.18.1

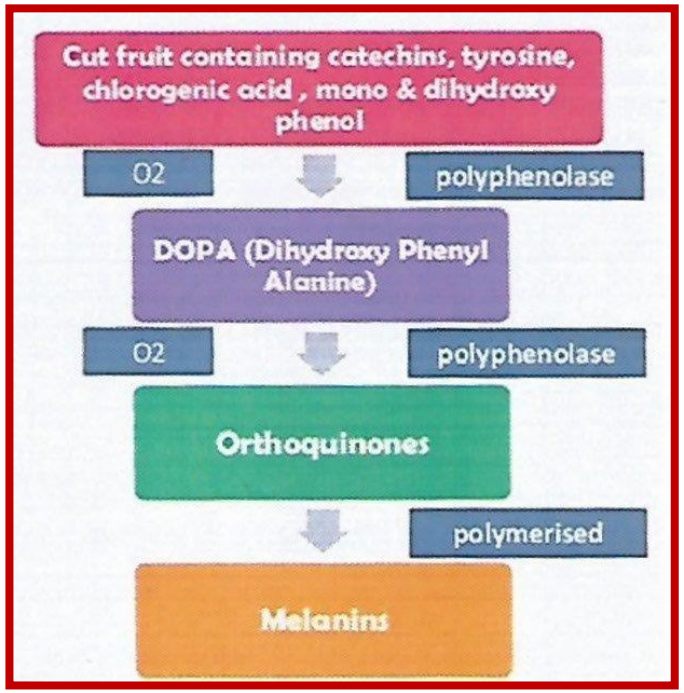
Accepted name: tyrosinase
Other name(s): monophenol monooxygenase; phenolase; monophenol oxidase; cresolase; monophenolase; tyrosine-dopa oxidase; monophenol monooxidase; monophenol dihydroxyphenylalanine:oxygen oxidoreductase; *N*-acetyl-6-hydroxytryptophan oxidase; monophenol, dihydroxy-L-phenylalanine oxygen oxidoreductase; *o*-diphenol:O_2 oxidoreductase; phenol oxidase
Comments: A type III copper protein found in a broad variety of bacteria, fungi, plants, insects, crustaceans, and mammals, which is involved in the synthesis of betalains and melanin. The enzyme, which is activated upon binding molecular oxygen, can catalyse both a monophenolase reaction cycle (reaction 1) or a diphenolase reaction cycle (reaction 2). So, it can catalyse both the monooxygenation of monophenols and the oxidation of catechols (like PPO).



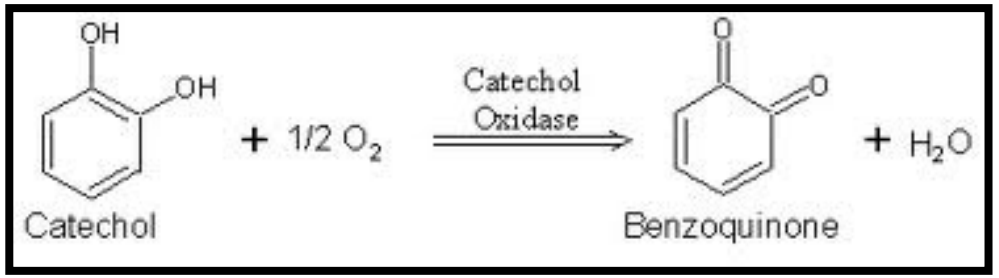
Different PPOs with different substrate specificity have been isolated from plants. Generally, PPO activity is very low in young plants.

Monophenol oxidase enzyme oxidizes mono-hydroxy-phenols to o-di-hydroxy-phenols. This enzyme is called **tyrosinase** in animals and sometimes called **crescolase** in plants.

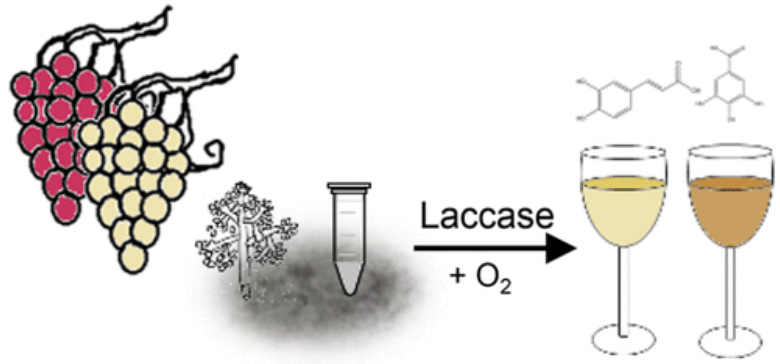




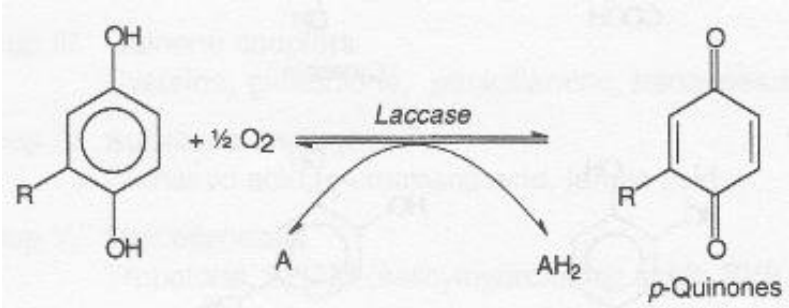
Diphenol oxidase enzyme converts diphenols to quinones. An example is the conversion of catechol to benzoquinone.



EC 1.10.3.2



Accepted name: laccase
Other name(s): urishiol oxidase; urushiol oxidase; *p*-diphenol oxidase
Comments: A group of multi-copper proteins of low specificity acting on both *o*- and *p*-quinols, and often acting also on aminophenols and phenylenediamine. The semiquinone may react further either enzymically or non-enzymically.



Laccase enzyme oxidizes p-diphenols. Basically, laccases oxidize substrates whose structure is similar to p-diphenols. The resulting quinones polymerize to form dark brown melanoidin pigments.

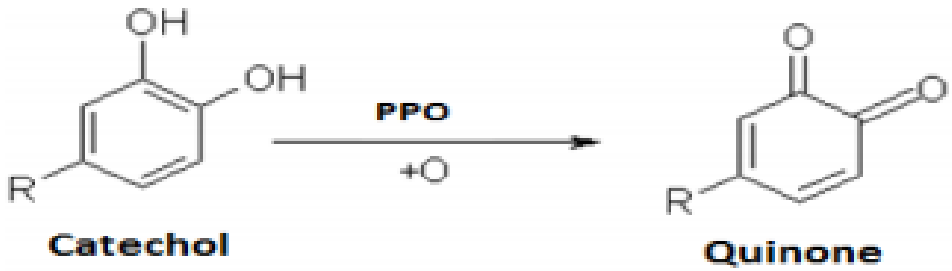


Figure 1: Polyphenol oxidase (Catechol oxidase, EC 1.10.3.1) enzyme reaction showing diphenol oxidase activity found mostly in plants. R in the figure represents any functional group

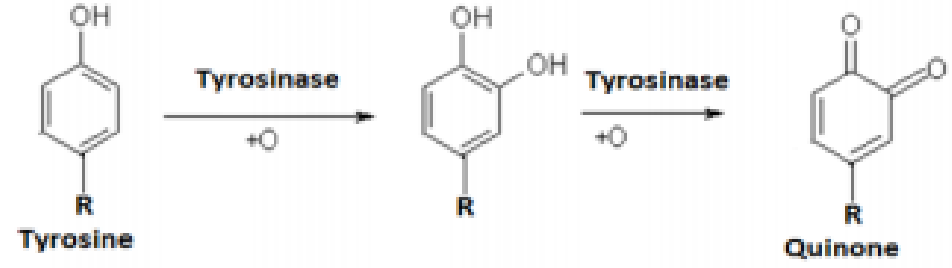


Figure 2: Polyphenol oxidase (tyrosinase, EC 1.14.18.1) showing both monophenol oxidase and diphenol oxidase activities found mostly in animals and fungi. R in the figure represents the functional group

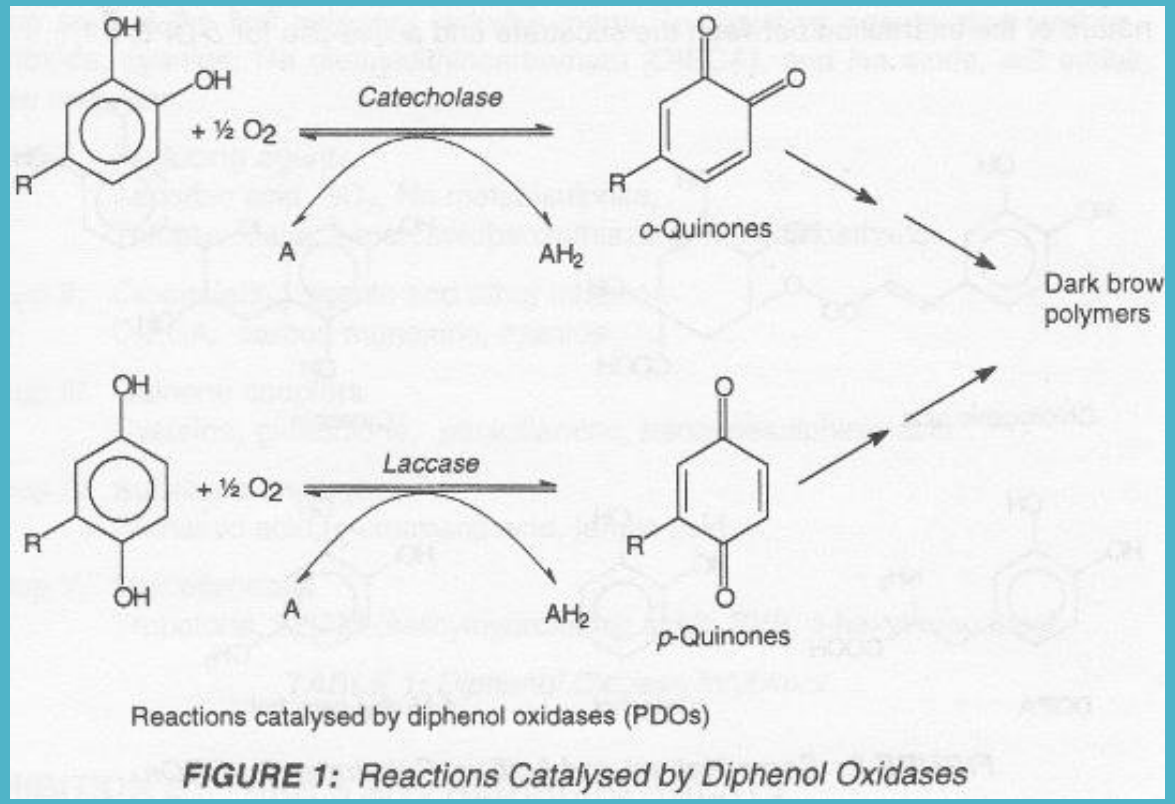
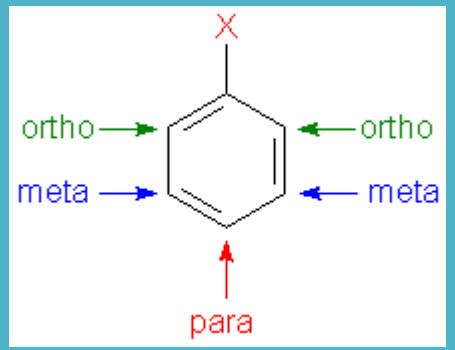
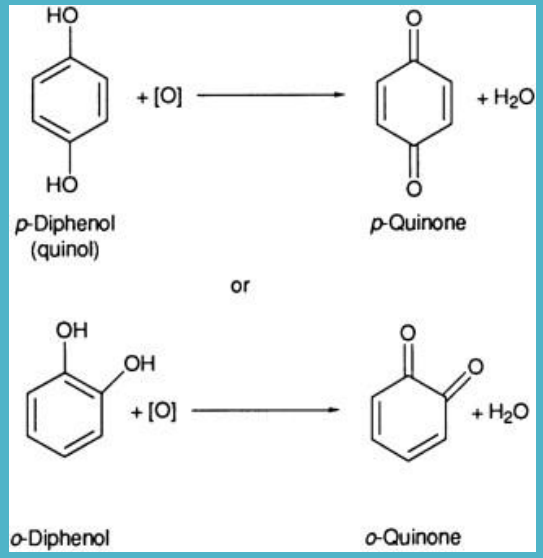


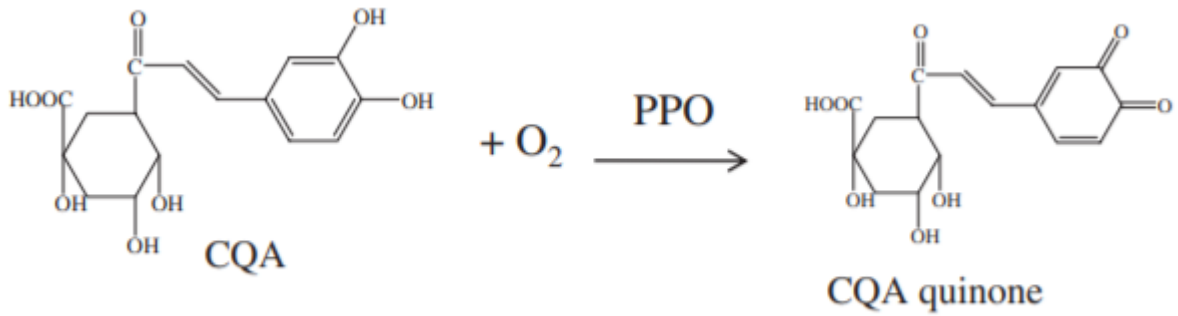
***FIGURE 1:** Reactions Catalysed by Diphenol Oxidases*





In EB, especially in animal tissues, the main substrate is *tyrosine*. In vegetal tissues, although phenolic acids and flavonoids (calculated to be 8000 in total) are phenolic compounds, the role of all of them in EB is not the same. The most important phenolic acid in terms of EB is chlorogenic acid. Among flavonoids, flavonols (campherol, quercetin, myricetin) are also very common in nature and play a role in EB reactions.

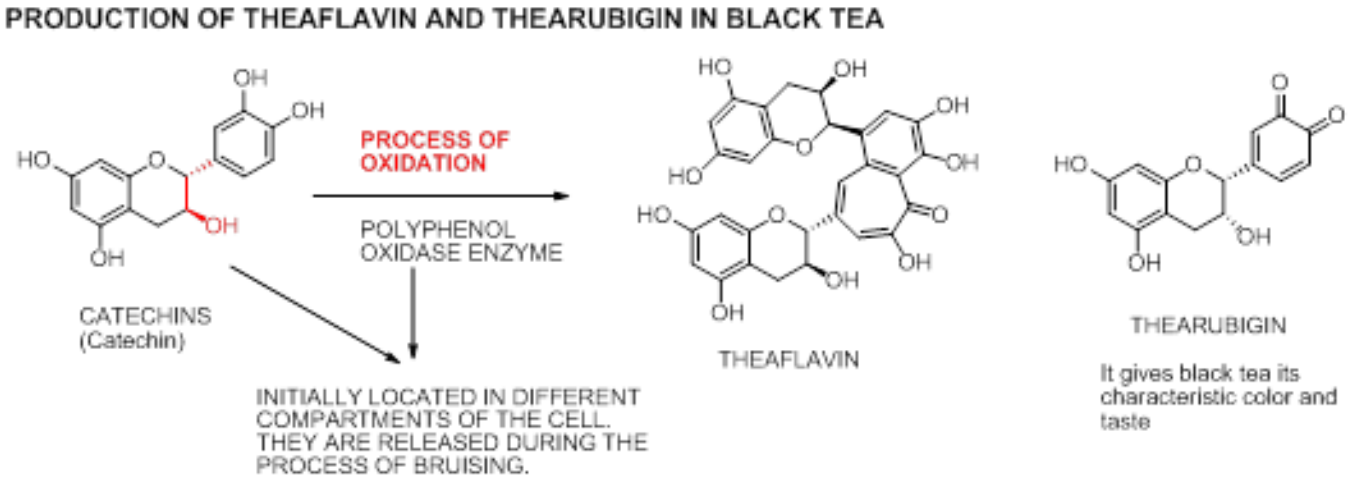
Chlorogenic acid (CQA) is one of the major polyphenols in apple and a good substrate for the polyphenol oxidase (PPO) in apple.



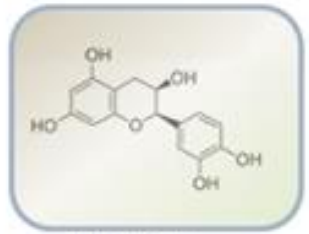
CQA

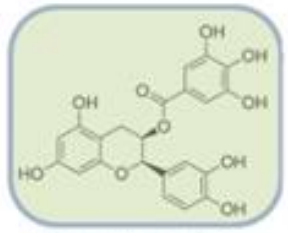
CQA quinone

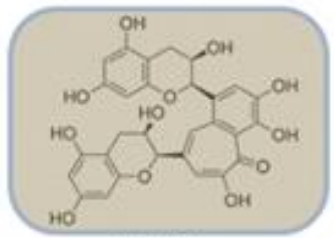
Among the flavonoids in EB, flavan 3-ols (catechins) are the most important substrate. In the production of black tea, these are especially oxidized with PPOs to form theaflavin and tearubigin polymers. On the contrary, in green tea production, this reaction is prevented by inhibiting PPOs.

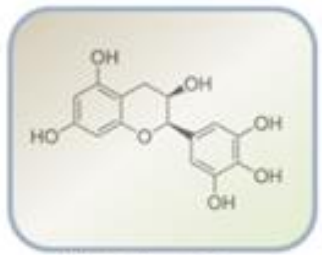


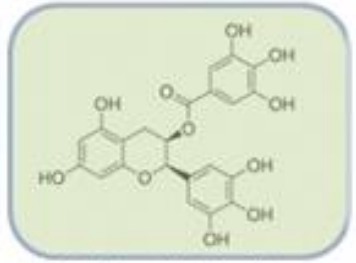
Major Flavonoids Found in Black and Green Tea

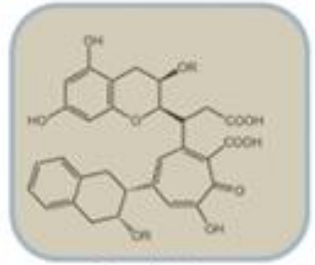

(–)-Epicatechin


(–)-Epicatechin-3-gallate


Theaflavin


(–)-Epigallocatechin

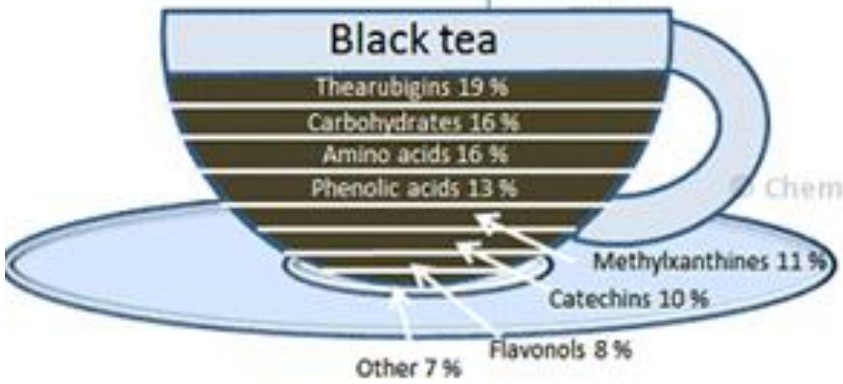

(–)-Epigallocatechin-3-gallate

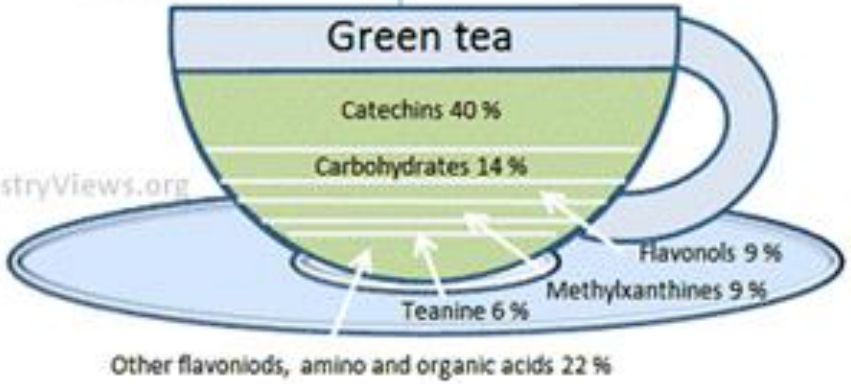

Thearubigin

R=Galloyl group

Leaves crushed and oxidized for 3–4 h. Oxidation converts simple catechins into polymeric flavonoids – theaflavins and thearubigins.

Leaves steamed before crushing to deactivate enzymes that catalyze the oxidative polymerization of catechins.





The main PPO substrates in some foods are:

Apple: Chlorogenic a., Caffeic a., P-coumaric a., Flavonol glycosides

Apricot: Isochlorogenic a., Caffeic a., Catechin, epicatechin

Cocoa: Catechins, leucoanthocyanidins, anthocyanins, complex phenols

Coffee: Chlorogenic a., Caffeic a.

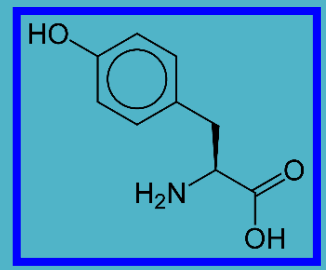
Lettuce: Tyrosine, caffeic a., Chlorogenic acid and its derivatives Tyrosine

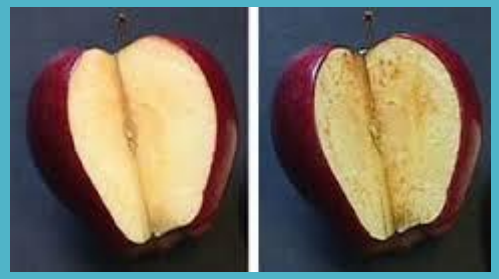
Mushrooms: Tyrosine, catechol, dopamine, adrenaline, noradrenaline

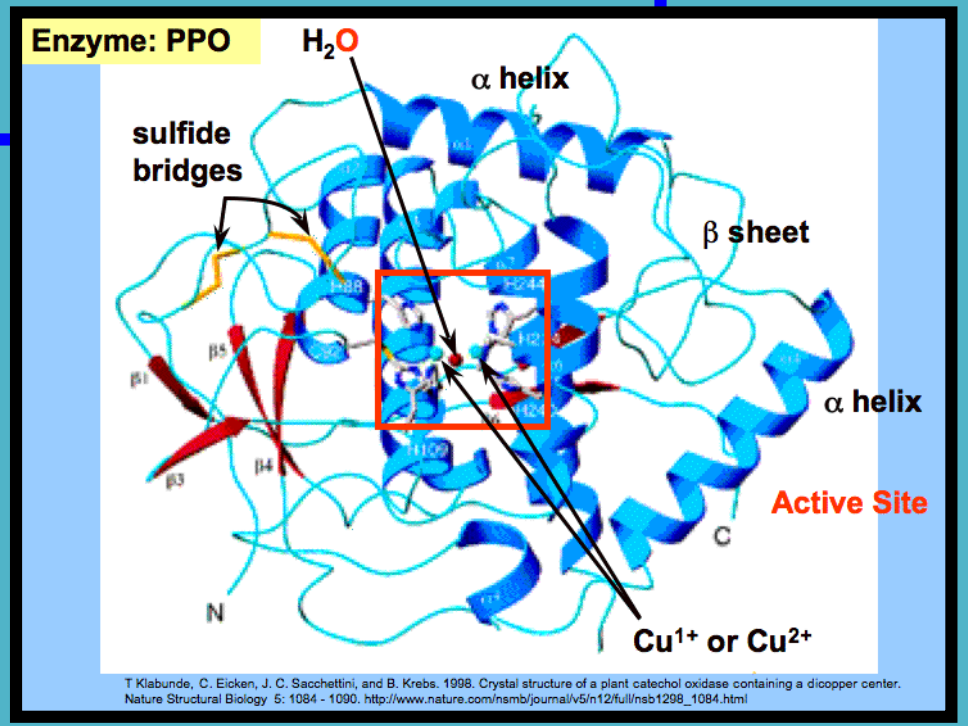
Lobster, Shrimp: Tyrosine

Potato: Chlorogenic a., Caffeic a., Catecol, p-cresol

Tea: Flavanols, catechins, tannins, cinnamic acid derivatives



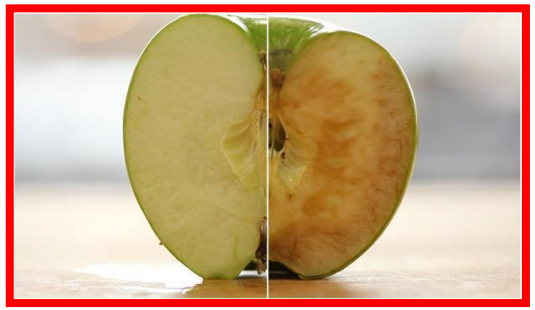




The molecular weight of PPO in plants varies between 57-63 kDa.

Rate of reaction to EE:

depends on pH, temperature, active PPO level, phenolic compound level and oxygen amount in tissue.



Enzymatic Browning

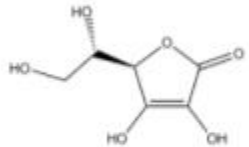
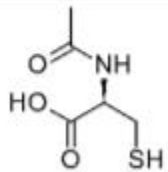
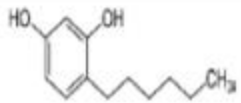
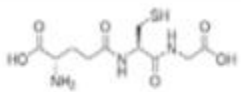
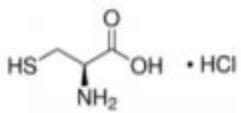
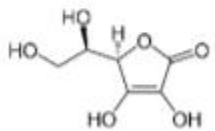
Acid

Blanching

Procedure		Enzyme inhibition
Heat Application Boiling in steam or water (70-105˚C), Pasteurization (60-85˚C) **Cooling** Preservation in cold, freezing		**Chelators** Sodium azide, Citric, malic, tartaric, oxalic, succinic acids, carbon monoxide, Some salts ($CaCl_2$, NaCl), ascorbic acid Sorbic acid, polyphosphates Macromolecules (proteins, polysaccharides) EDTA
Physical Methods Drying (Freeze-drying, spray-drying, sun-drying, microwave-drying)	**Chemical Methods** Salts such as sodium, sugars such as sucrose, glycerol, propylene glycol, modified corn syrup	**Aromatic Carboxylic Acids** Benzoic acids, cinnamic a. Aliphatic Alcohols Peptides and Amino Acids
Radiation		**Honey**
High Pressure (600-900Mpa)		**Proteases**
Supercritical CO_2 (58 atm, 43^{0}C)		**Acidifying agents (citric, malic, tartaric)**
Ultrafiltrations		**Chitosan**
Ultrasound		

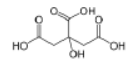
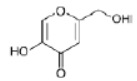
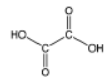
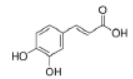
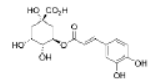
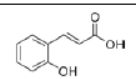
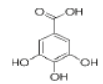
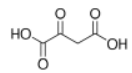
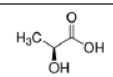
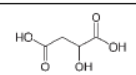
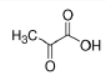
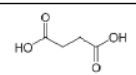
SUBSTRATE INHIBITION		PORDUCT INHIBITION
Removal of Oxygen	**Removal of Phenols**	**Reducers** Sulphides, ascorbic acid derivatives, Thiol-containing compounds such as cytesine
Vacuum	Building complex (Cyclodextrins, sulphated polysacc., Chitosan)	
Soaking in water, syrup, brine	Enzymatic modification (o-methyltransferase)	Amino a., peptides, proteins
Reducers (ascorbic a., BHA, BHT, TBHQ, PG)		Chitosan
		Maltol

Table Antioxidant effects of chemical compounds.

Compound	Structure	Conc.[1]	Product	Effect
Ascorbic acid		5 mM 0.3 mM	Apple juice	Reducing oxidant substrates Reduction of *o*-quinones to their precursor diphenols
N-acetyl cysteine		1.7 mM 25 mM	Potato Apple	Competitive inhibition of PPO [2] Reactive oxygen species scavenger
4-Hexylresorcinol		1.8 µM	Peer Apple	PPO inactivation Synergistic inhibition with ascorbic acid and N-acetyl cysteine
Glutathione		0.08%	Peer Apple juice	Inhibited PPO activity
Cysteine hydrochloride		1.8 µM 1%	Fruit salad	Inhibited PPO activity
Erythorbic acid		19.6 µM	Fruit salad	Inhibited PPO activity Oxygen scavenger

[1] Conc.: concentration; [2] PPO: polyphenoloxidase.

Table Chelating agents and acidulants of chemical compounds.

Compound	Structure	Conc. [1]	Product	Effect
Citric acid		2.7 mM	Lettuce-head	PPO [2] noncompetitive inhibitor
Kojic acid		25 µM	Apple Potato	Strong chelator such as Fe(III) and Cu(II) Inactivated PPO enzyme (bind to Cu in PPO)
Oxalic acid		2.0 mM 10 µM	Apple Lettuce	Chelating copper from the active site of PPO PPO noncompetitive inhibitor
Caffeic acid		955.7 µM	Apple juice Unripe grapes juice	Low inhibitory activity on enzymatic browning
Chlorogenic acid		1 mM	Loquat juice	Prevention of enzymatic browning through inactivating PPO
Coumaric acid		50 µg/mL	Potato Apple puree	Inhibited PPO activity
Gallic acid		59.2 µM	Unripe grapes juice	Low inhibitory activity on enzymatic browning
Carboxylic acid		1%	Apple	Inhibitory effects on enzymatic browning due to metal-chelating activities or lowering pH
Oxaloacetic acid		1%	Apple	Inhibitory effects on enzymatic browning due to metal-chelating characteristics or lowering pH
Lactic acid		1%	Apple	Inhibitory effects on enzymatic browning because of their metal- chelating characteristics or lowering pH
Malic acid		163.8 mM	Unripe grapes juice	Inhibitory effects on enzymatic browning because of their metal-chelating characteristics or lowering pH
Pyruvic acid		1%	Apple	Inhibitory effects on enzymatic browning due to metal-chelating characteristics or lowering pH
Acetic acid		0.1%	Lettuce-head Cabbage	No apparent effect on PPO activity
Succinic acid		536.7 mM	Unripe grapes juice	Less effective in controlling enzyme browning
Formic acid		1%	Apple	Less effective in controlling enzymatic browning Inhibitory effects on enzymatic browning because of their metal-chelating characteristics or lowering pH

[1] Conc.: concentration; [2] PPO: polyphenoloxidase.

Food Chemistry: Course Summary

Water

Molecular properties: The water molecule; Hydrogen bonding; water organization
Physicochemical properties: Phase changes; Water-solute interactions; Solution properties; pH and pK_a values, Water activity; Glass transitions
Analysis: Methods to determine amount and type of water

Carbohydrates

Origin and Use: Biological origin; Food Applications
Molecular properties: Monosaccharides, Oligosaccharides, Polysaccharides; Nomenclature; Glycosidic bonds; Polymer structure
Chemical Reactions: Oxidation, Reduction, Maillard, Caramelization
Physicochemical properties: Denaturation; Aggregation; Surface Activity
Functional properties: Sweeteners; Humectants; Thickening agents; Gelling Agents; Emulsifiers
Examples: Starch, Cellulose, Pectin, Alginate, Carrageenan, Xanthan, Guar.
Analysis: Methods to determine amount and type of carbohydrates

Proteins

Origin and Use: Biological origin; Food Applications
Molecular properties: Amino acids (polar, non-polar, ionic); Peptide bonds
Physicochemical properties: Denaturation; Aggregation; Surface Activity
Functional properties: Enzyme activity; Thickening agents; Gelling Agents; Emulsifiers; Water holding
Examples: Dairy, Egg, Meat, Plant Proteins
Analysis: Methods to determine amount and type of proteins

Lipids

Origin and Use: Biological origin; Food Applications
Molecular properties: Fatty acids; Acyl Glycerols; Phospholipids; Cholesterol/Phytosterols; Structure, nomenclature
Chemical Reactivity: Oxidation; Hydrogenation; Interesterification
Physicochemical properties: Crystallization; Fractionation; Rheology; Emulsion formation
Functional properties: Organoleptic; Health; Structural (plasticity)
Examples: Plant, Animal, Fish
Analysis: Methods to determine amount and type of lipids