The Chemistry of Honeybees

Part 1

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Substances produced or secreted by Honeybees

Beeswax ←
Pheromones
Venom
Royal Jelly
Honey ←

Substances Gathered and Used by the Honeybee

Nectar ←
Pollen ←
Propolis
Water

Some of the Chemicals used by the Beekeeper to care for Honeybees

Terrimycin
Apistan (Fluvalinate)
Coumaphos
Paradichlorobenzene
Fumidil B
Sugar Esters (Sucrose Octanoate)
Chloramphenicol (Chloromycetin)---Illegal Antibiotic

The Chemistry Of Nectar & Honey

Nectar

The total Sugar Concentration of Nectar varies from
4 to over 80% (w/v) or (grams/100 milliliters of Nectar)

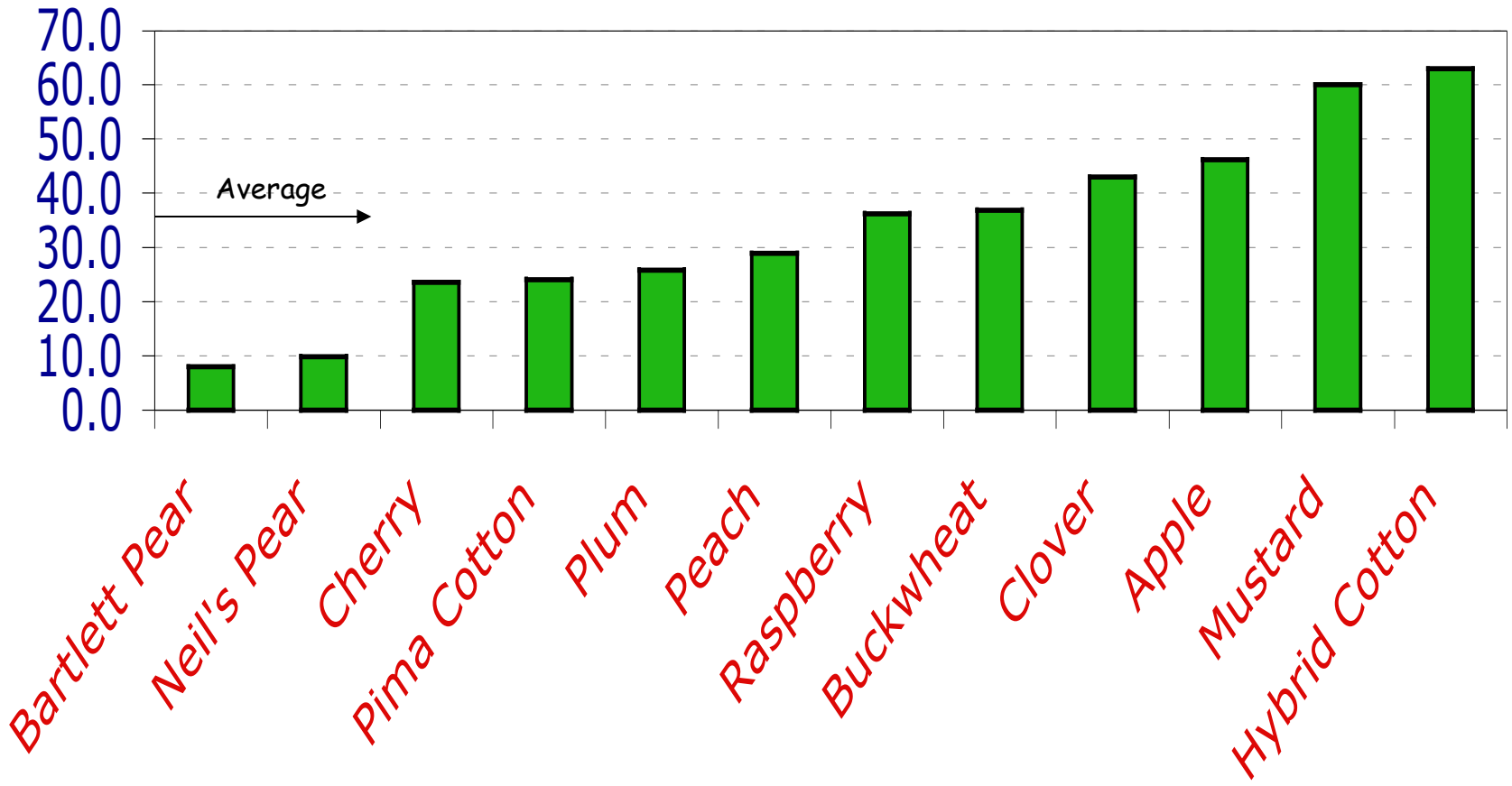
Bees rarely forage for nectar with sugar concentrations lower than 15%
(unless absolutely necessary for survival, although it puts a strain on the colony)

Average: 30-40% Sugar or 60-70% Water

Since honey contains 87-98% sugar, bees must eliminate an average of 47 to 68% of the water contained in nectar.

Along with Sugars, Nectar and Honey contain trace amounts of: Amino Acids (pollen), Free Organic Acids, Minerals, Vitamins, Enzymes, Esters, Ketones, Aldehydes, Alcohols, Lipids, & Beeswax & more

Average % Sugars in some Nectars



Feeding Bees: (Sugar Concentrations)

Spring: 2 parts water
1 part sugar

100 ml water + 50 dry ml of Sugar
50 dry ml of Sugar = 43.8 g
Final Volume = 129 ml
Grams of Sugar/100 ml = 34.0%

Fall/Winter: 1 part water
2 parts Sugar

50 ml water + 100 dry ml of Sugar
100 dry ml of Sugar = 87.6 g
Final Volume = 98 ml
Grams of Sugar/100 ml = 89.4%

One bee usually gathers more nectar than it can process itself. It passes the nectar to other bees.

How is water decreased from nectar to honey?

1. Water is absorbed out of the honey sacs of the bees and the concentration of sugars increases----dialysis
2. After storing nectar in cells, the bees drive off water by air movement and heat----desiccation

Supersaturation

Honey is a supersaturated sugar solution

This means the sugar concentration in honey is higher than what it would be naturally without the help of honeybees. Maximum amount of sugar in the smallest volume of liquid honey.

Physical Properties:

1. Honey is hygroscopic (hydrophilic) meaning it absorbs water readily if left uncovered.

   For this reason honeybees cap honey cells to prevent water absorption.

   a. Spoilage due to bacterial/fungal contamination.

   b. Fermentation from yeasts.

   Paenibacillus larvae (American foulbrood): forms a spore and can survive the hygroscopic nature of honey.

2. Crystallization tends to occur in a supersaturated solution.

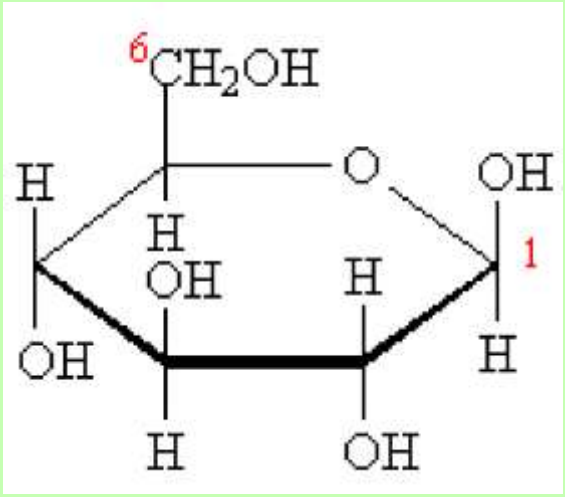
   Glucose (one of the 3 main sugars in honey) spontaneously precipitates out by losing water (becoming glucose monohydrate) and takes the form of a solid.

   a. Granulated Honey (natural crystallization).

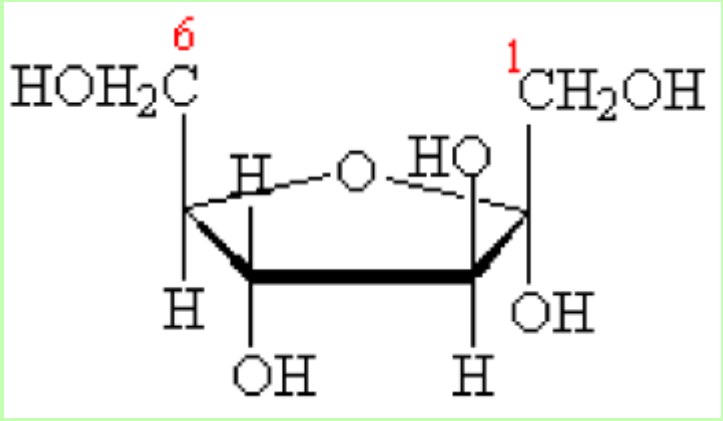
   b. Creamed Honey (controlled crystallization).

Sugars (Carbohydrates) in Honey

Monosaccharides



Isomers



Glucose (Dextrose)
Ave. 31%

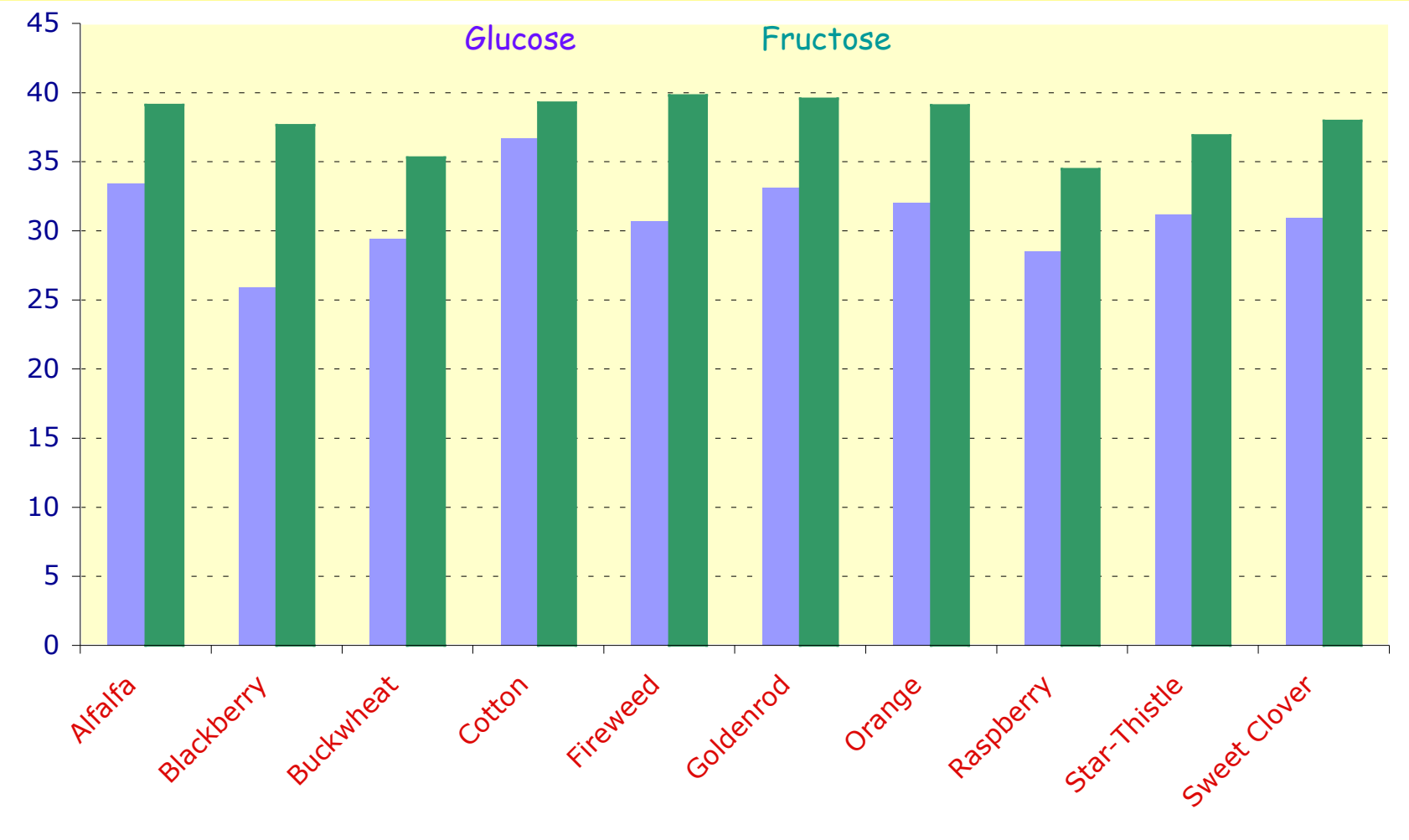
Fructose (Levulose)
Ave. 38%

$C_6H_{12}O_6$

Glucose ⟷ Fructose

Glucose or Fructose Isomerase

Relative % of Glucose and Fructose in Various Honeys

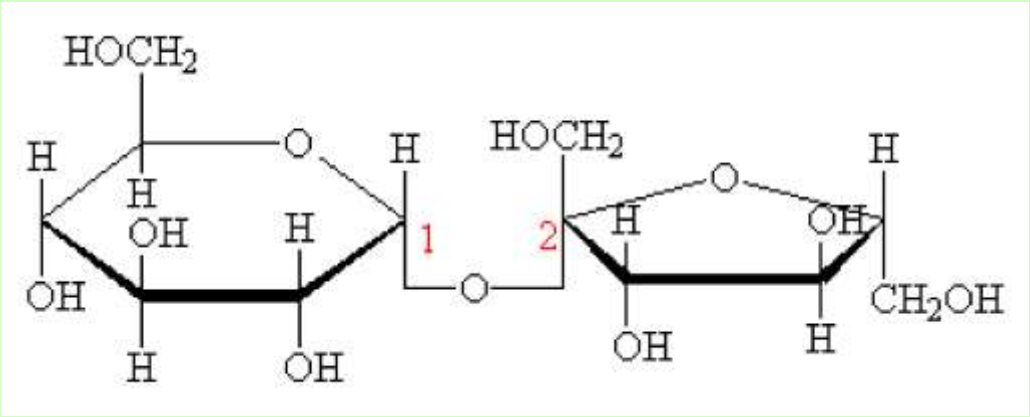


The Glucose and Fructose concentrations in Honey do not vary greatly from one variety to another

Sugars (Carbohydrates) in Honey

Disaccharides (Ave. 9%)

Ave. 1%



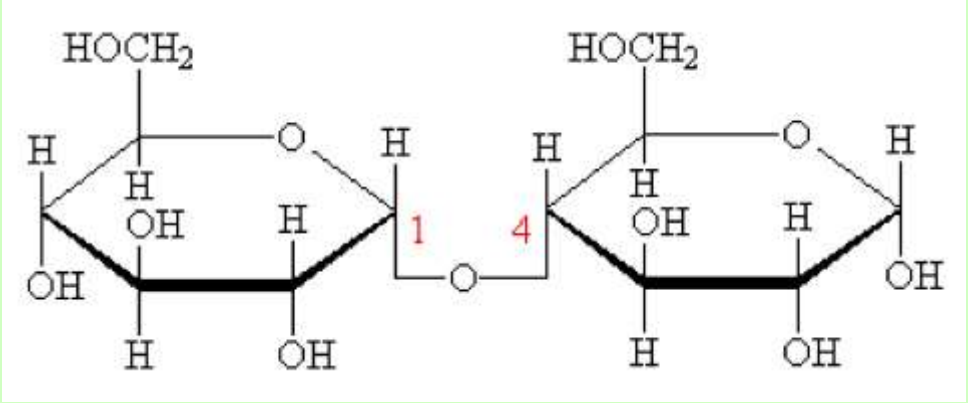
Glucose Fructose

Sucrose (Table Sugar)

$C_{12}H_{22}O_{11}$

Isomers

Ave. 7%

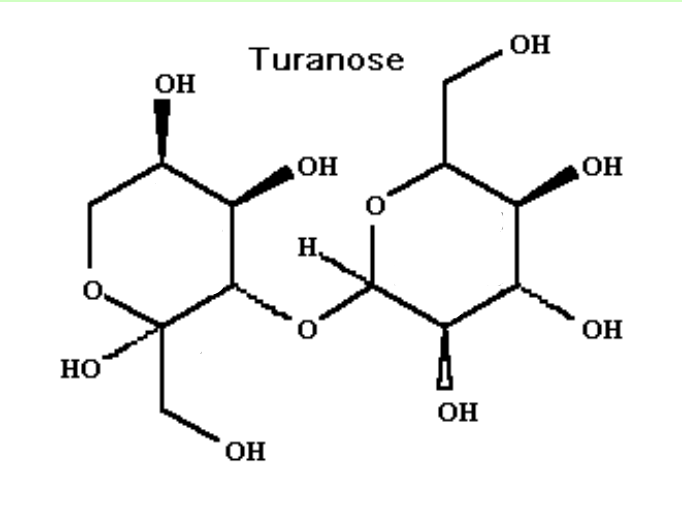


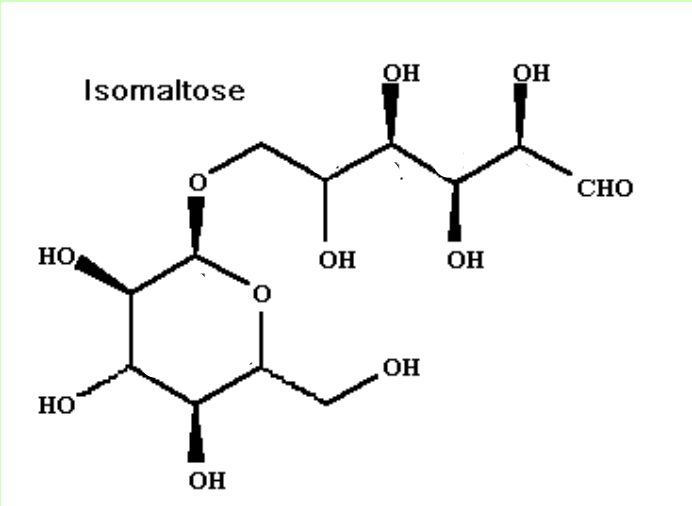
Glucose Glucose

Maltose

Other Disaccharides in Honey

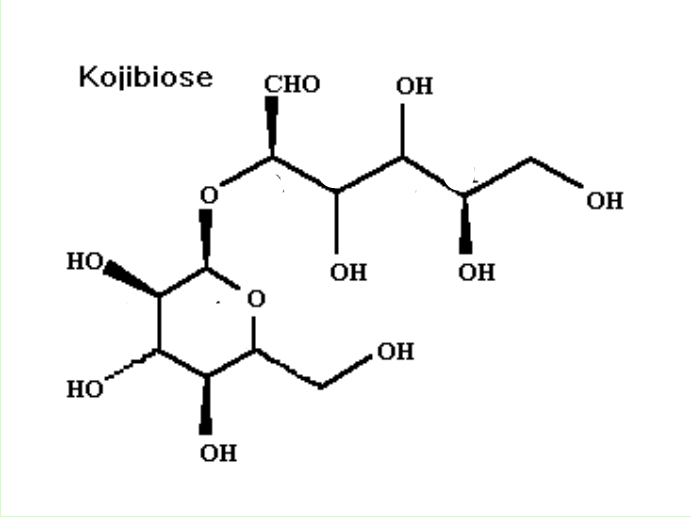
~1%

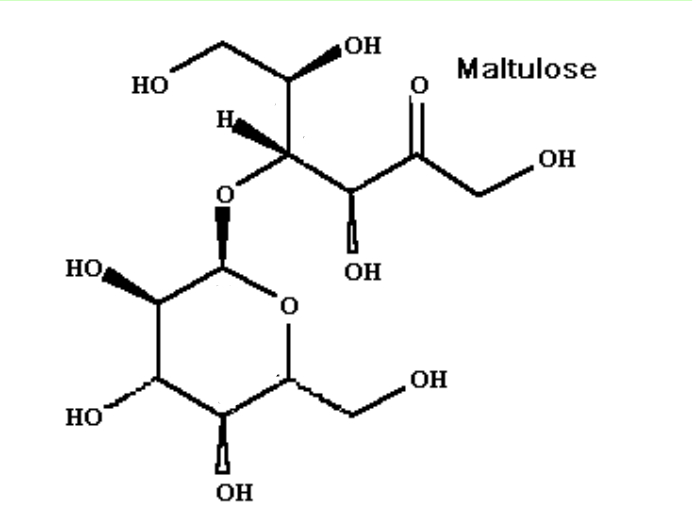




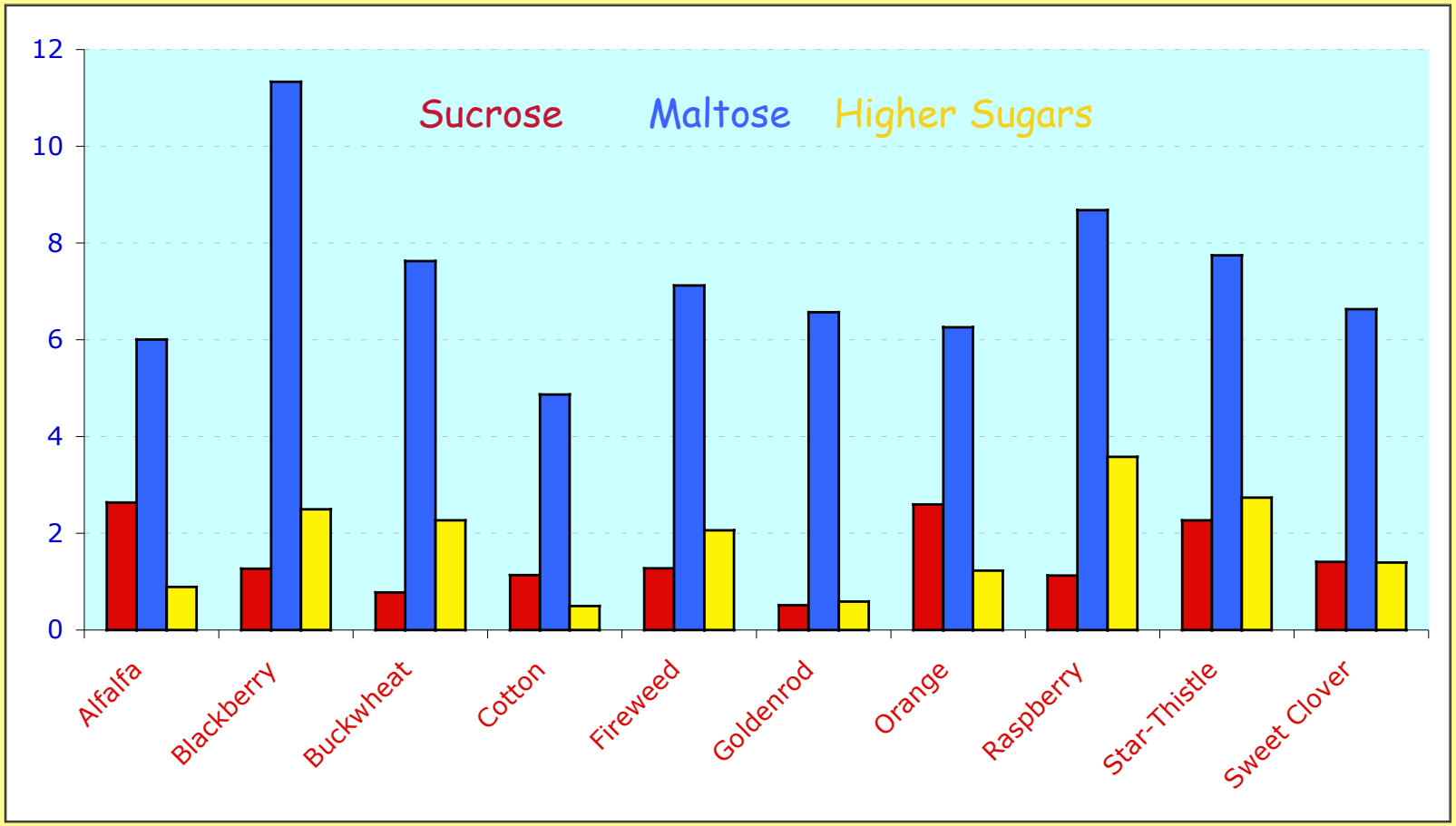
$C_{12}H_{22}O_{11}$

Isomers



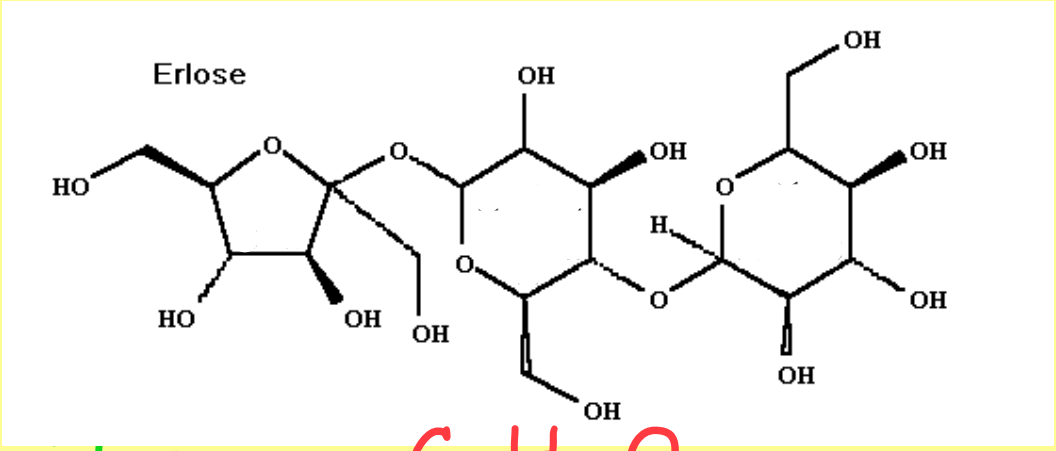


Relative % of Sucrose, Maltose and Higher Sugars in Various Honeys



Higher Sugars = Oligosaccharides

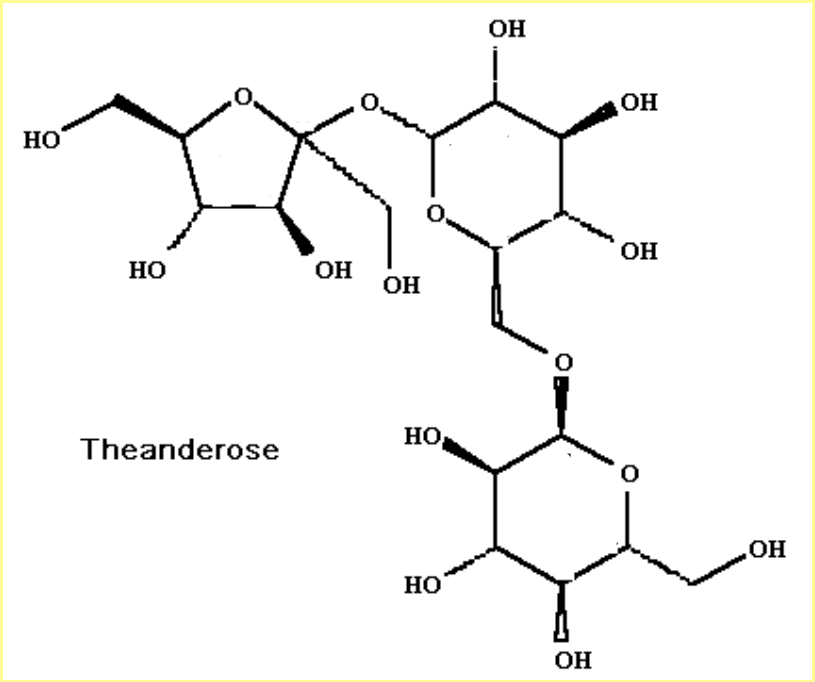
Some Oligosaccharides in Honey (Ave. 4.2%)

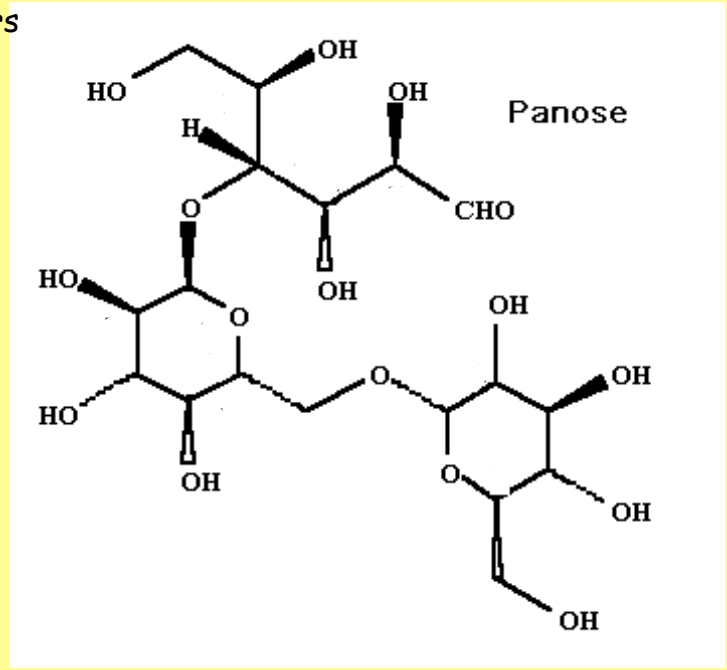


Trisaccharides

$C_{18}H_{32}O_{16}$

Isomers





Enzymes in Honey: Invertase (Sucrase)

Invertase is protein secreted by the honey bee from it's salivary gland into the honey sac.
This enzyme catalyzes (helps) the breakdown of sucrose into glucose and fructose

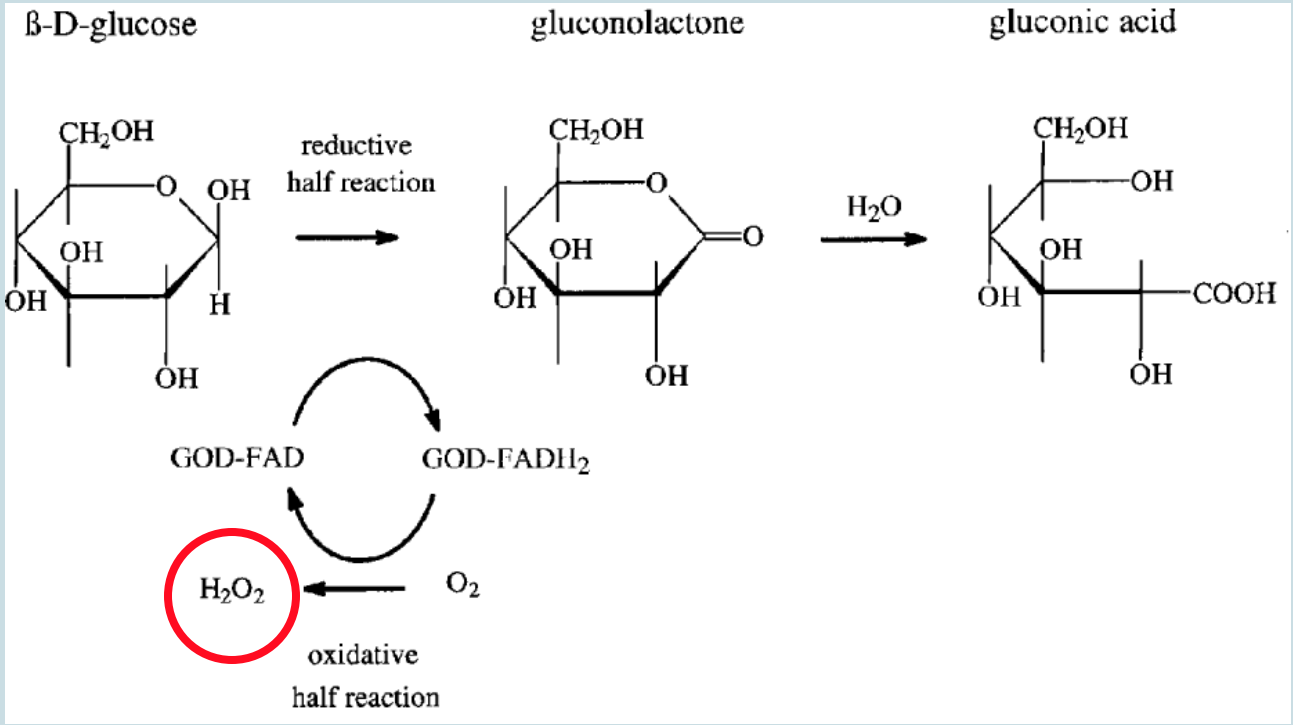
$$\text{Sucrose} + O_2 \xrightarrow{\textit{Invertase}} \text{Fructose} + \text{Glucose}$$

This reaction of slightly reversible and Invertase also catalyzes the synthesis of a small amount of disaccharides and more complex carbohydrates
(Higher Sugars or Oligosaccharides).
Because of this there will always be some sucrose and higher sugars in honey.
Invertase in raw extracted honey will continue to breakdown sucrose and honey will ripen over a period of time. Heating will destroy invertase.

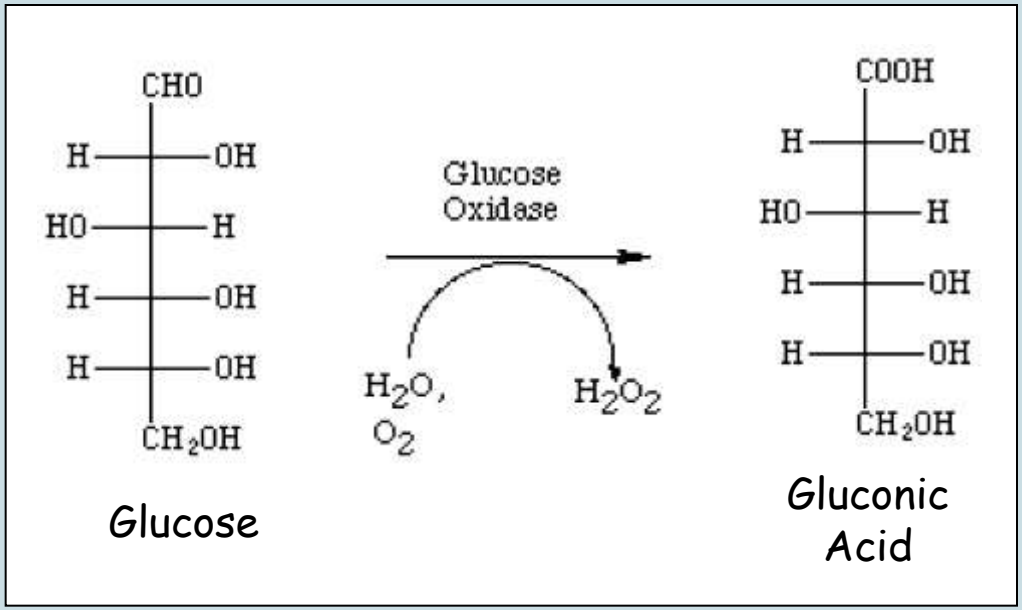
$$\text{Sucrose, Maltose Or Higher Sugars} \xleftarrow{\textit{Invertase}} \text{Fructose \& Glucose}$$

Enzymes in Honey: Glucose Oxidase

Glucose Oxidase another protein enzyme and is secreted from the hypopharyngeal glands of honeybees. This enzyme catayzes (helps) the breakdown of glucose to gluconolactone, which in turn converts to Gluconic Acid.



Glucose Oxidase activity helps in the preservation of honey by the production of Hydrogen Peroxide which inhibits the growth of bacteria, fungus and yeasts.



Glucose Oxidase

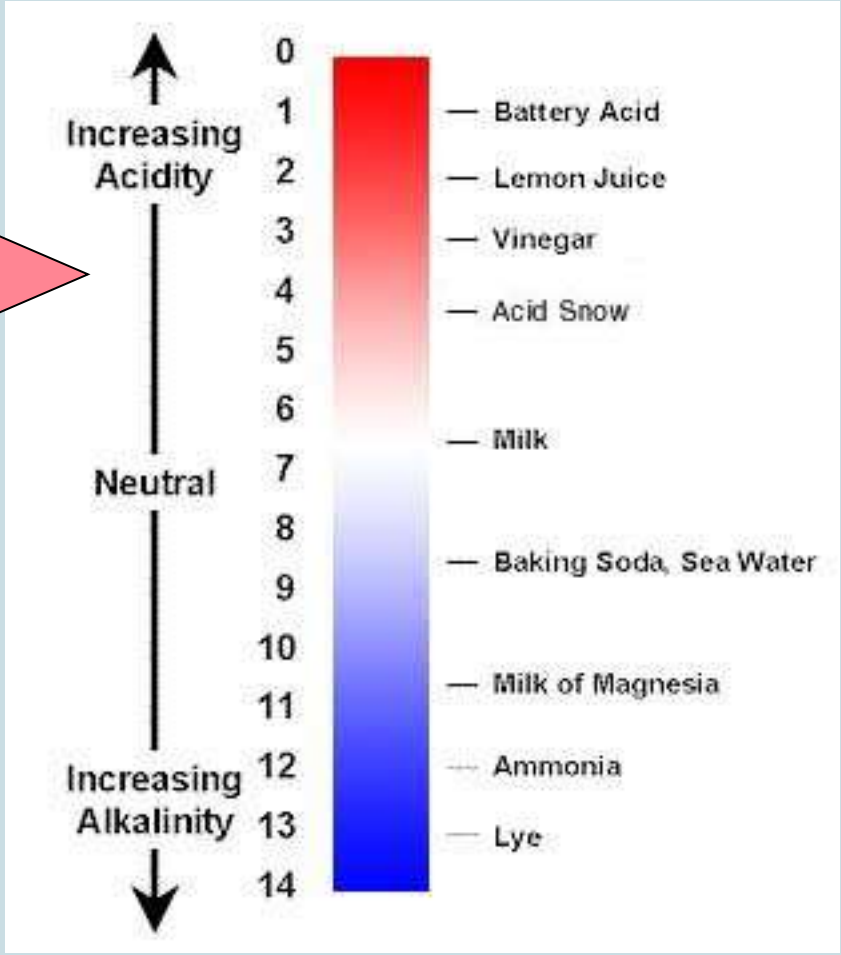
Gluconic Acid and other carboxylic or organic acids in honey account for the acid pH (3.2 To 4.5) and also contribute to the different tastes in various types of honey.

Honey

Gluconic acid (70-80% of all free acids), Acetic Acid, Butyric Acid, Citric Acid, Formic Acid, Lactic Acid, Malic Acid, Oxalic Acid, Succinic Acid, Fumaric Acid, a-Ketoglutaric Acid, Pyroglutamic Acid, and Maleic Acid

The acidic nature of honey has an inhibitory effect on many pathogens.

The sweetness of honey offsets the acidic nature of honey



The Metabolism or (Catabolism) Of Glucose

Why do honeybees need glucose all year round?

How is glucose used by the honeybee (and by other organisms)?

How do honey bees maintain a temperature in the hive above 60° F (16° C) and the brood chamber from 91° F (32° C) to 97° F (35 ° C) all year?

The Metabolism (Catabolism-burning up) of Glucose by Honeybees

Glucose is the primary sugar used for the production of ATP (Adenosine Triphosphate), which is a high energy chemical used by the honeybee (and most living creatures) for the production of heat and energy

The metabolism or catabolism of glucose into heat and energy (ATP) is accomplished in 3 stages:

1. Glycolysis of Glucose:

Glycolysis of glucose takes place in the cytoplasm of the each cell.

This process converts glucose into pyruvate, a chemical readily usable to the honey bee in the biochemical pathways involved in energy production.

Pyruvate is a 3 carbon molecule, so for every molecule of glucose there is produced 2 molecules of pyruvate:

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P \longrightarrow 2\,\text{Pyruvate} + 2NADH + 2ATP + 2H^+$$

This representative reaction actually represents the outcome of 10 different reactions, each involving a specific enzyme.

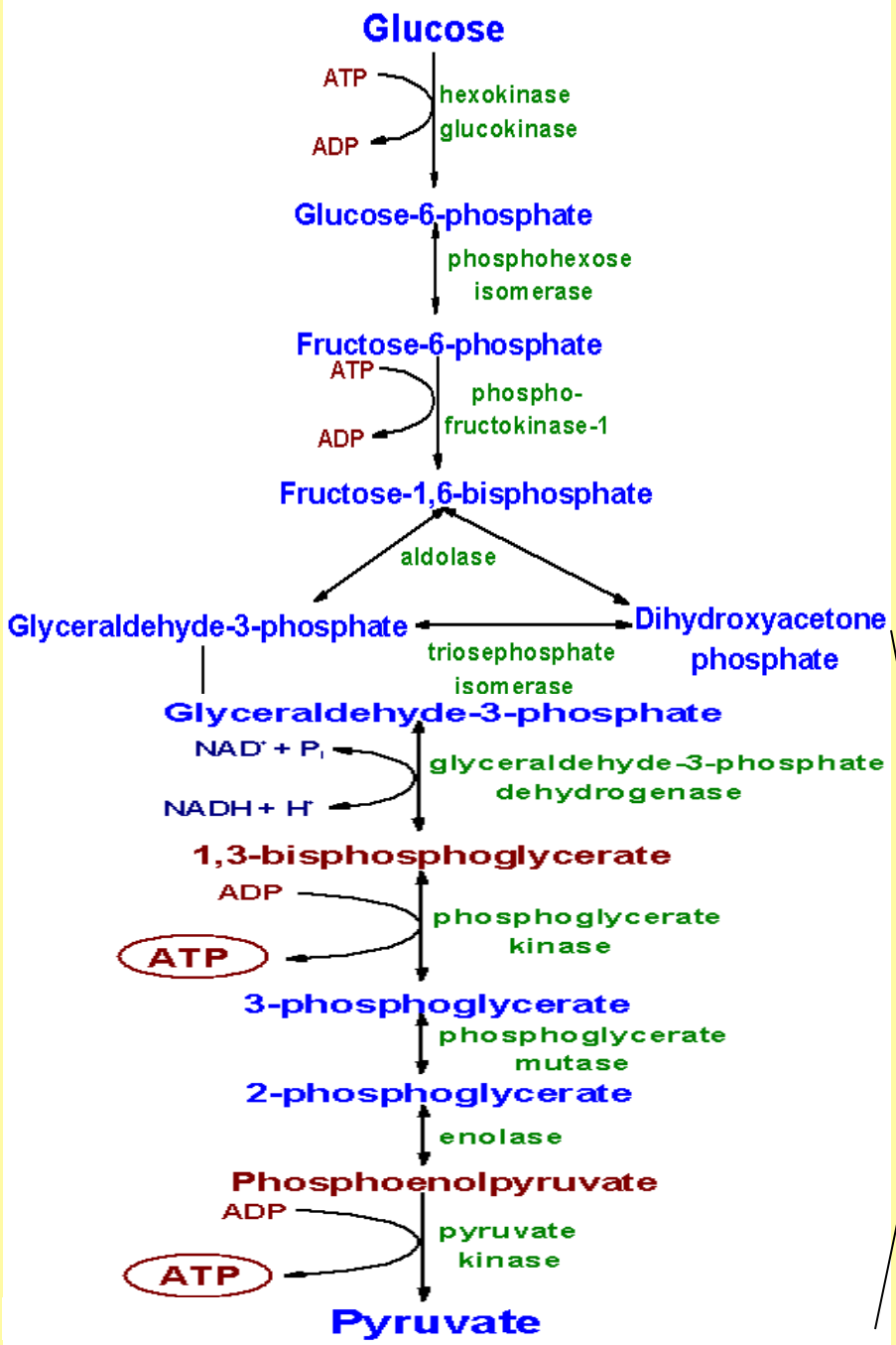
Glucose + 2NAD$^+$ + 2ADP + 2P

2 Pyruvate + 2NADH + 2ATP + 2H$^+$

Potential Energy

Stored Chemical Energy

Direct Heat & Energy



2. The Tricarboxylic Acid Cycle (Kreb Cycle or Citric Acid Cycle):

Within the cytoplasm of honeybee cells, pyruvate molecules couple with Co-enzyme A and enter into the Tricarboxylic Acid Cycle or Kreb Cycle (also called the Citric Acid Cycle). The reactions within this cycle are exothermic and heat is produced which the honey bee uses to keep the hive at a constant warm temperature. Excess energy is stored chemically in reduced molecules of NADH (Nicotinamide adenine dinucleotide) and $FADH_2$ (flavin adenine dinucleotide). These molecules are high in stored chemical energy and are transported to the mitochondria of each cell.

$$\text{Pyruvate} + \text{CoA} \quad \text{NAD}^+ \longrightarrow \text{Acetyl-CoA} + CO_2 + \text{NADH}$$

$$\text{Acetyl-CoA} + 3\text{NAD+} + \text{FAD} + \text{GDP} + \text{P} \longrightarrow 2CO_2 + \text{CoA} + 2\text{NADH} + FADH_2 + \text{GTP}$$

Nine separate equilibrium reactions occur to convert one molecule of pyruvate into heat and stored chemical energy in the form of NADH and $FADH_2$; each facilitated by an enzyme catalyst.

Pyruvate + CoA NAD^+

Acetyl-CoA + CO_2 + NADH

Potential Energy

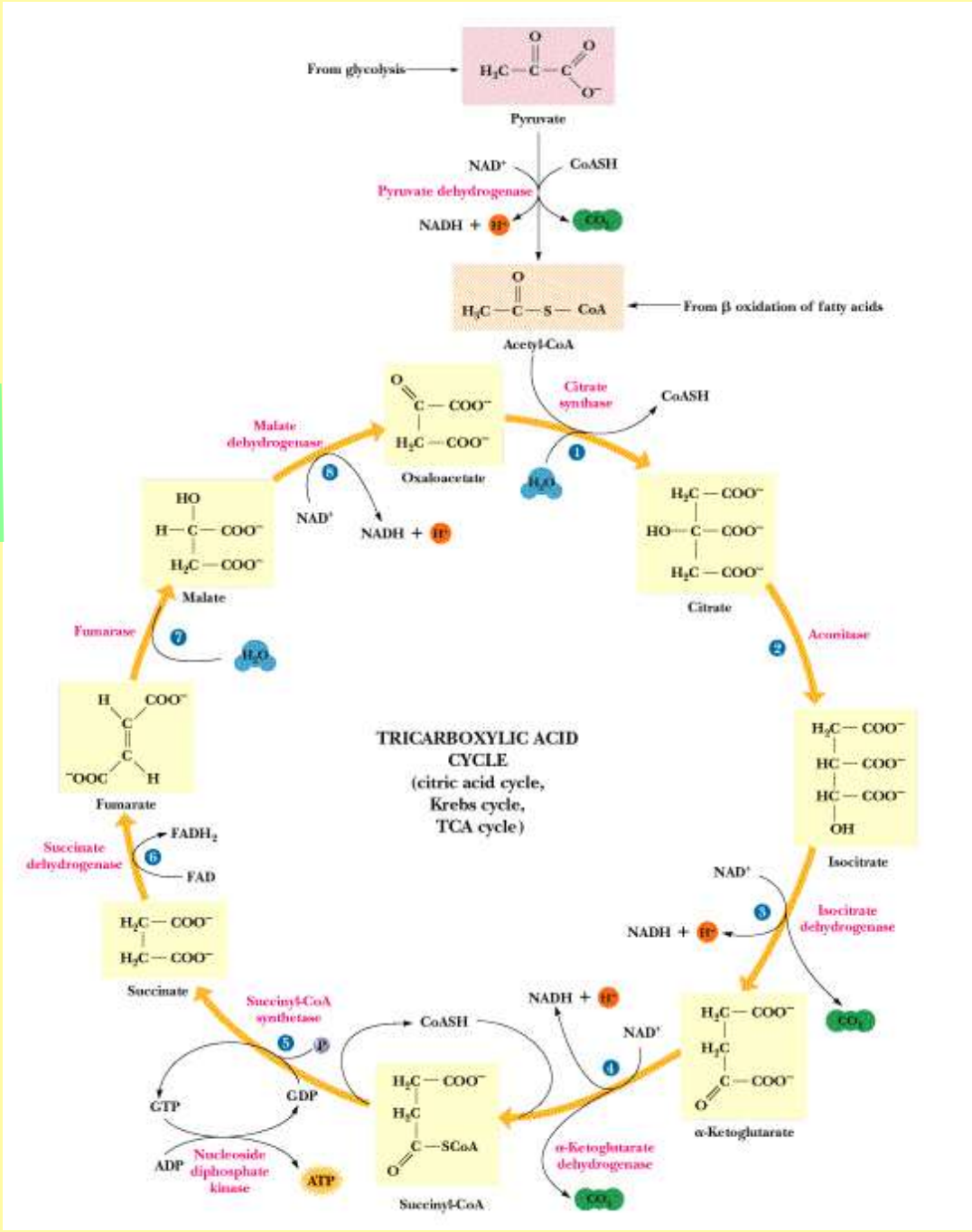
Stored Chemical Energy

Acetyl-CoA + 3NAD+ + FAD + GDP + P

$2CO_2$ + CoA + 2NADH + $FADH_2$ + GTP → ATP

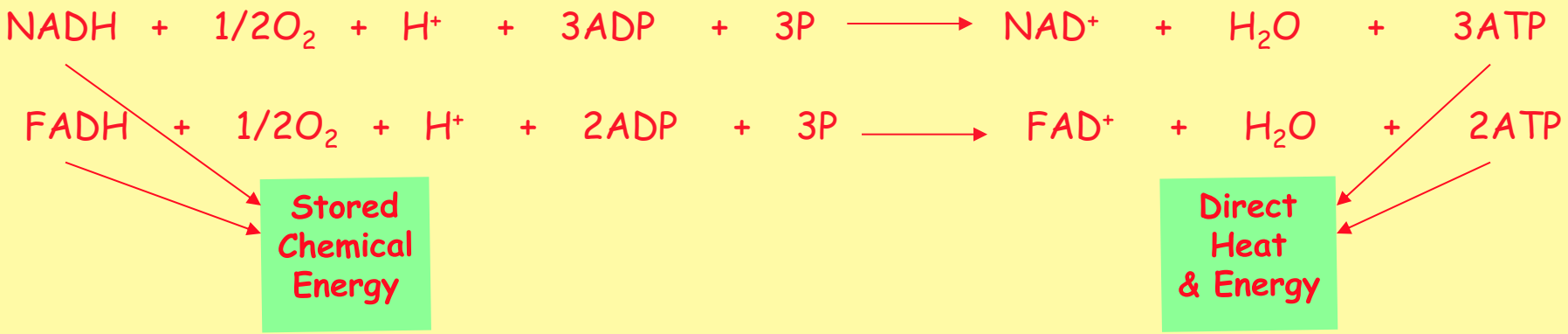
Stored Chemical Energy

Direct Heat & Energy



3. Oxidative Phophorylation:

This final step in the conversion of glucose into energy is accomplished in the mitochondria of each cell. Here the produced NADH and FADH2 release their stored chemical energy by converting it into ATP which is directly used by the cells to allow the bee to move, metabolize other reactions (synthesis of beeswax, pheromones, etc.), fly, etc.



There are 36 ATP molecules produced for every glucose molecule used by the honey bee.

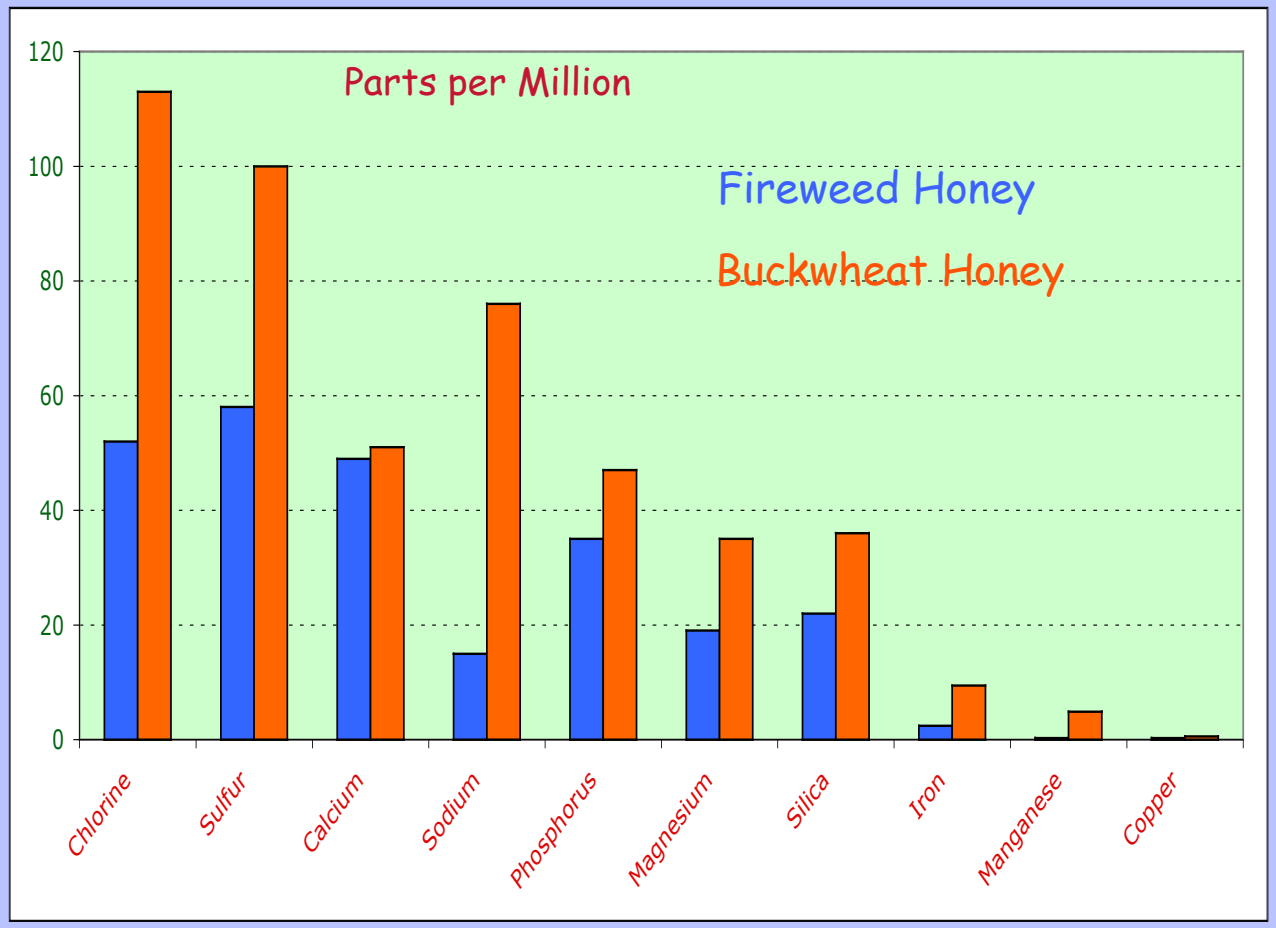
During movement and flight, this ATP is used as fuel.

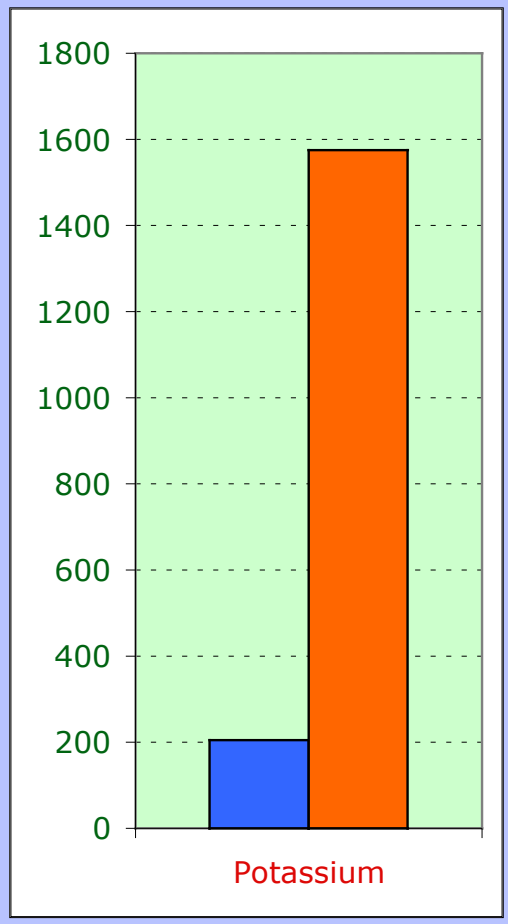
It has been found that the metabolic rate derived from glucose metabolism in the honeybee wing muscle is 3 times as efficient as the rate in the wing muscles of hummingbirds and 30 times as efficient as in active human muscles.

Fireweed Honey

Comparison of the Mineral Content Between Water White Honey & Extra Dark Amber Honey

Buckwheat Honey





The Chemistry Of Pollen

Pollen consists of: Average Amounts

	%		ppm
Protein	23.7	Iodine	Trace
Carbohydrates	27.0	Fluoride	Trace
Lipids	4.8	Selenium	Trace
Phosphorus	0.53	Thiamine	Trace
Potassium	0.58	Niacin	Trace
Calcium	0.23	Riboflaven	Trace
Magnesium	0.15	Pyridoxine	Trace
Sodium	0.04	Pantothenate	Trace
	ppm	Folic Acid	Trace
Iron	140	Biotin	Trace
Manganese	100	Vitamin B12	Trace
Zinc	78	Vitamin C	Trace
Copper	14	Vitamin A	Trace
Nickel	4.5	Carotenes	Trace
Boron	Trace	Vitamin D	Trace
Chromium	Trace	Vitamin E	Trace
Molybdenum	Trace	Vitamin K	Trace

Range

8 to 40% Protein

15-45% Carbohydrate

1-15% Lipids

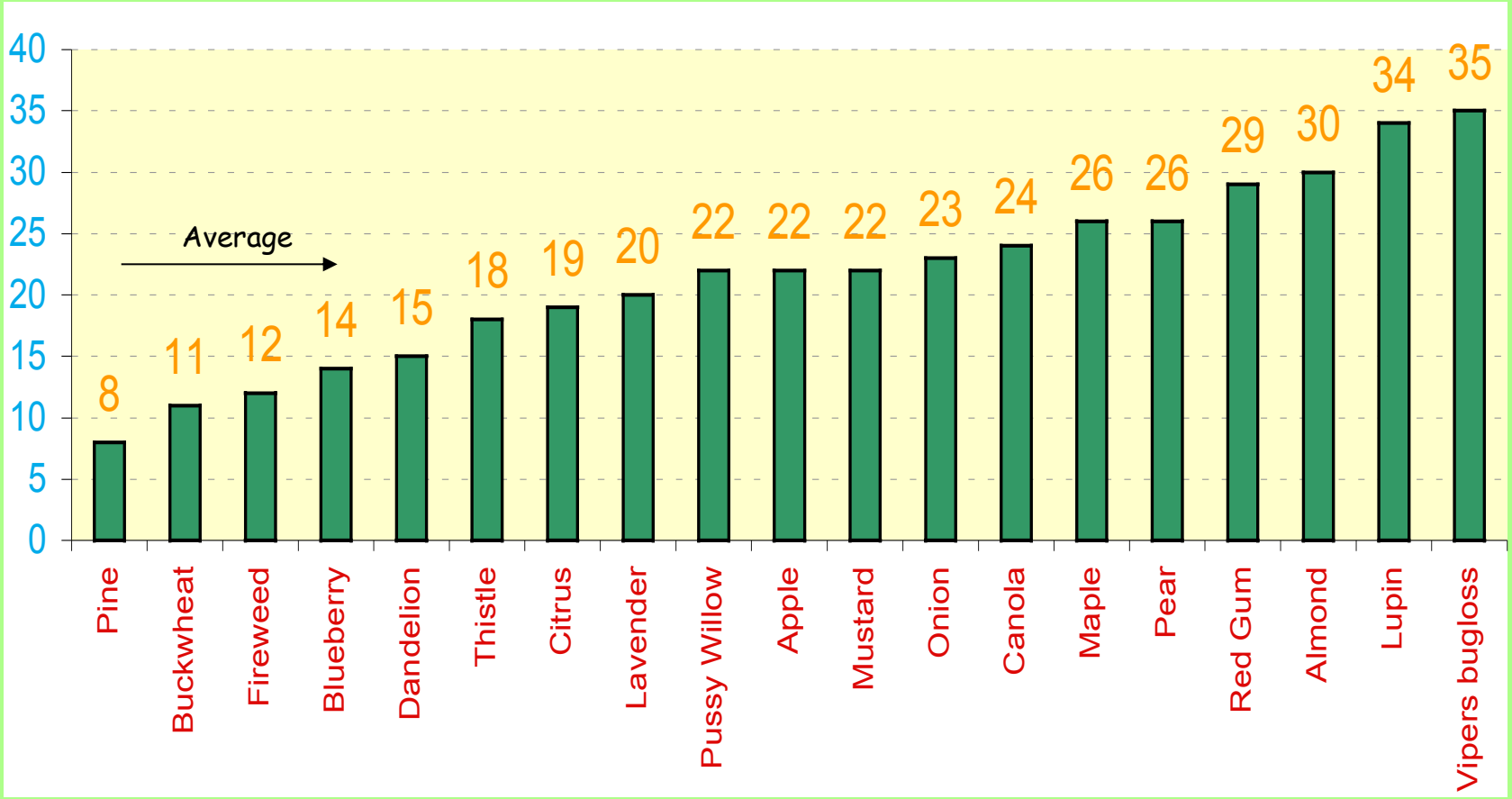
Entomophilous Pollen: insect-transferred
heavier, stickier an
more colorful in shades of yellow
orange, brown, black and red.

Anemophilous Pollen: wind-transferred
lighter, less colorful

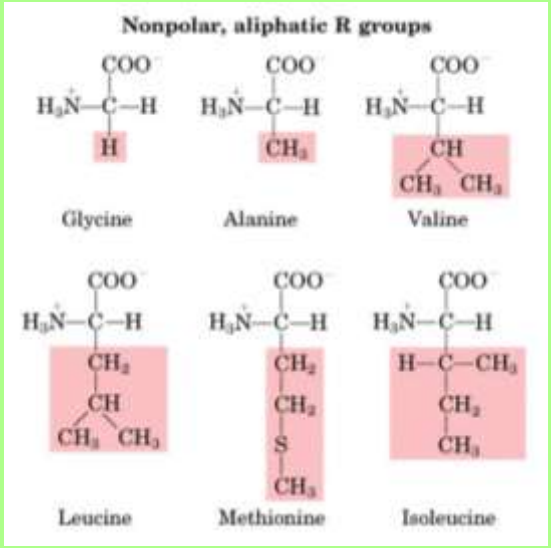
Honeybees get 100% of their protein for brood production and metabolism
from Natural Pollen, Pollen Substitutes or Pollen Supplements

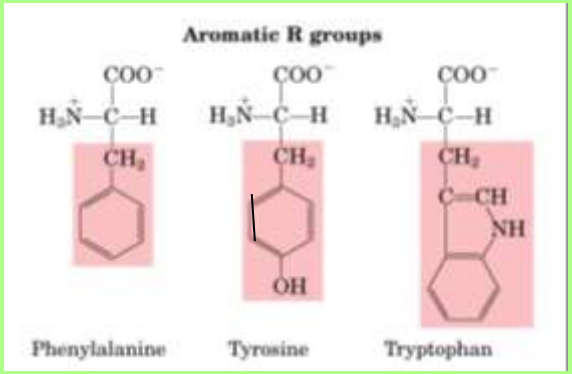
Pollen Supplement: Pollen Substitute + some Natural Pollen

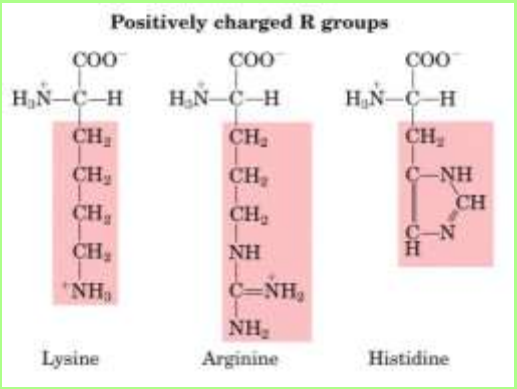
Percent of Crude Protein of Various Pollens

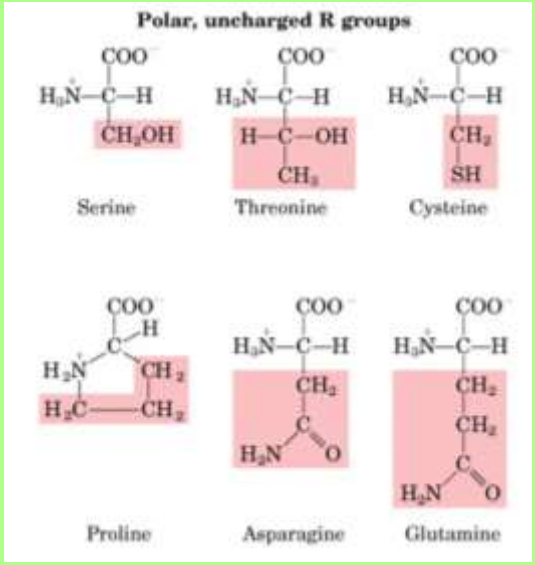


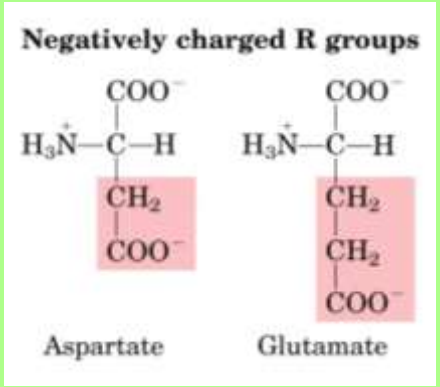
Amino Acids











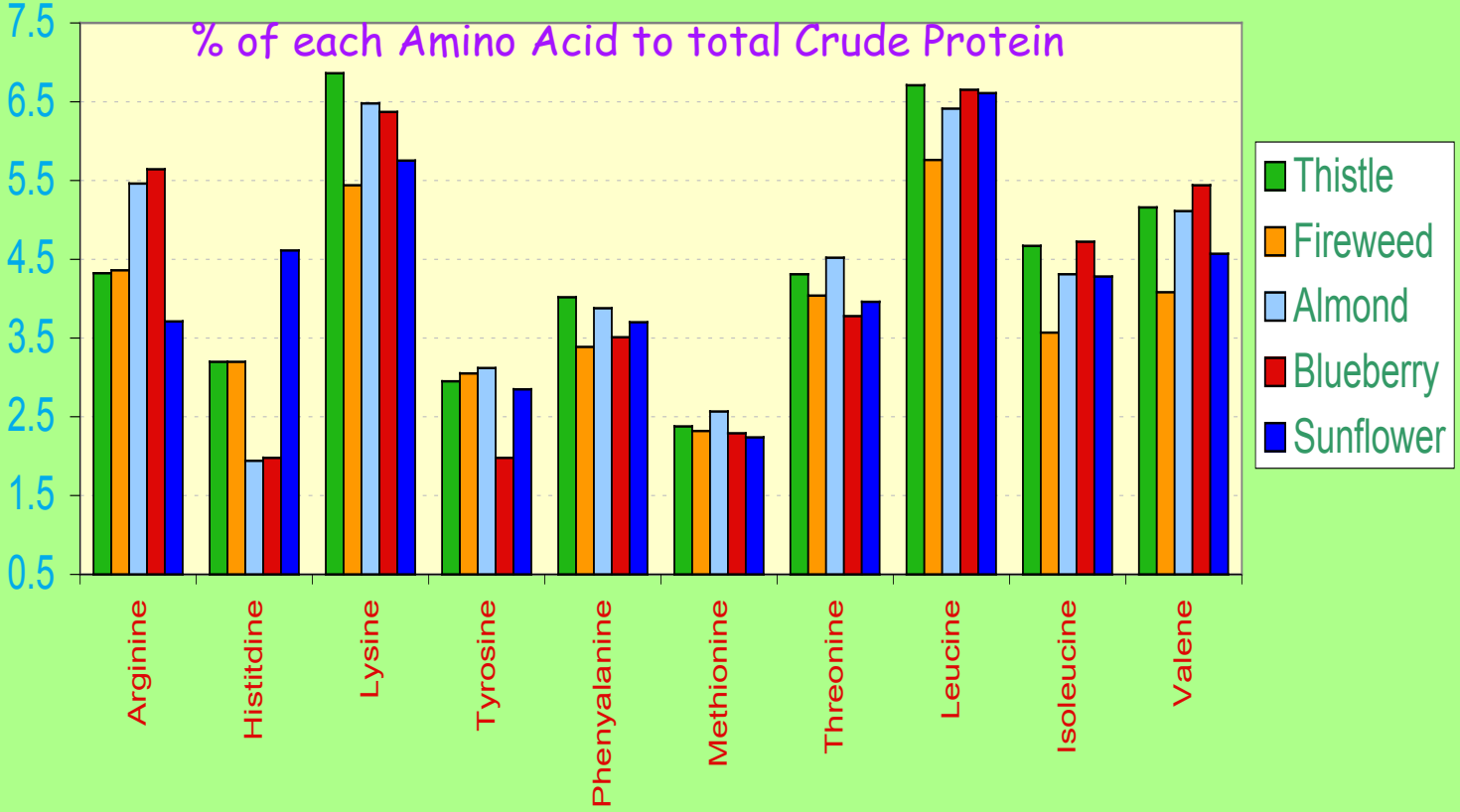
Aminoacetic Acid
Carnitine
GABA
Citrulline
Hydroxyproline
Cystine
Glutathione
Ornithine
Taurine

Judging pollen for quality:

High in % Crude Protein content: Fair 10%, Good 20% Very Good: >25%

High in the quantity of ten essential amino acids:

Arginine
Histidine
Lysine
Trytophane
Phenylalanine
Methionine
Threonine
Leucine
Isoleucine
Valine



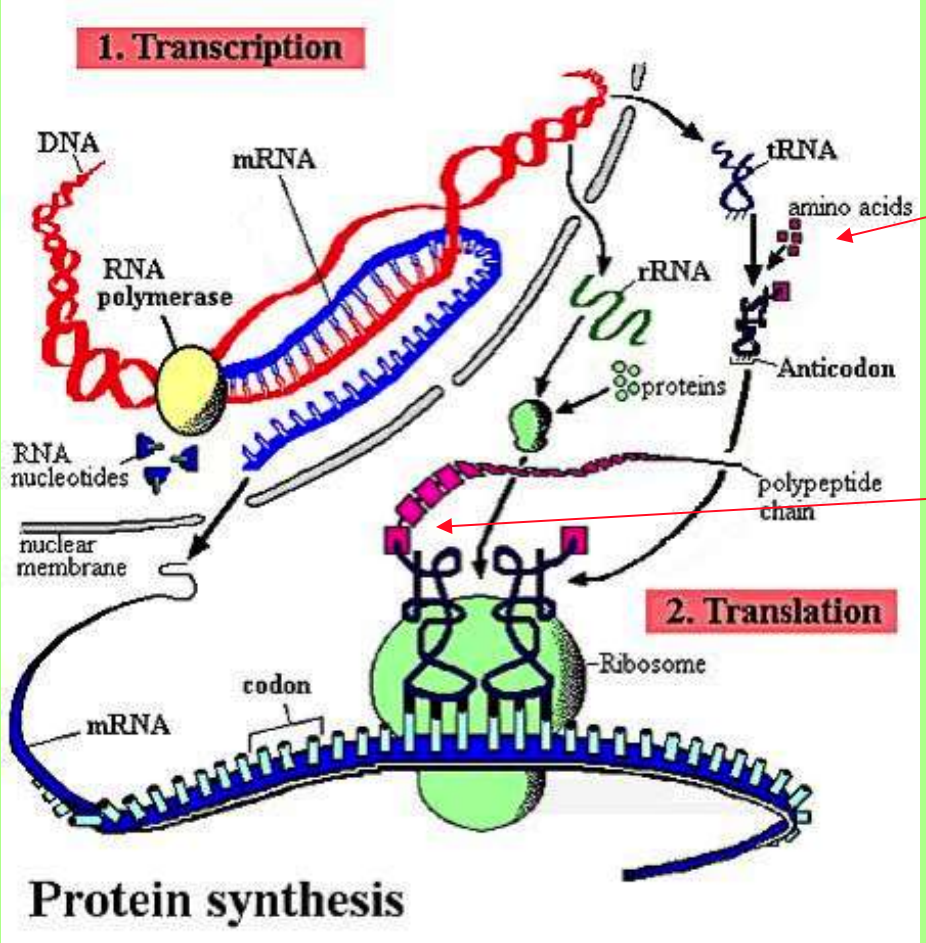
From the chart above you can see that pollen from blueberry flowers are sufficient in providing Arginine, Leucine, and Valine, but are defienciet in Histidine and Tyrosine. Sunflower pollen is sufficient in Histidine and Leucine, but defient in Arginine.

The Utilization of Pollen by Honeybees for Brood Production and Metabolism

Brood Production----Protein Synthesis from Amino Acids

Protein Synthesis occurs in the ribosomes of cells

All Amino Acids and especially the 10 essential Amino Acids MUST be randomly available for protein sysnthesis to occur,



Here all Amino Acids are needed randomly to allow proteins to be synthesized in the cells of honeybees and brood. Millions of different proteins are syntesized from amino acids from pollen.

Proteins are synthesized by connecting amino acids through polypeptide bonds. This is called translation of proteins and occurs in the ribosomes of honeybee cells.

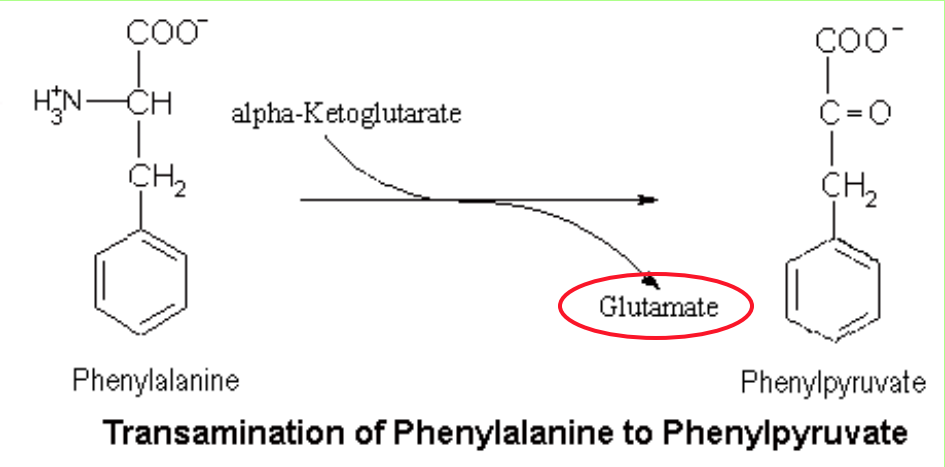
Amino Acid Metabolism (Catabolism)

Amino acids from pollen, like glucose from nectar, are also used by the honeybee to produce energy. The metabolism of amino acids involve 2 basic steps common to all the different amino acids:

1. Transamination of Amino Acids

In this step amino acids are converted to glutamate and Keto acids.

Amino Acid + a-ketoglutarate $\xrightarrow{\textit{transaminase}}$ Keto Acid + Glutamate



Transamination of Phenylalanine to Phenylpyruvate

Potential Energy

Stored Chemical Energy

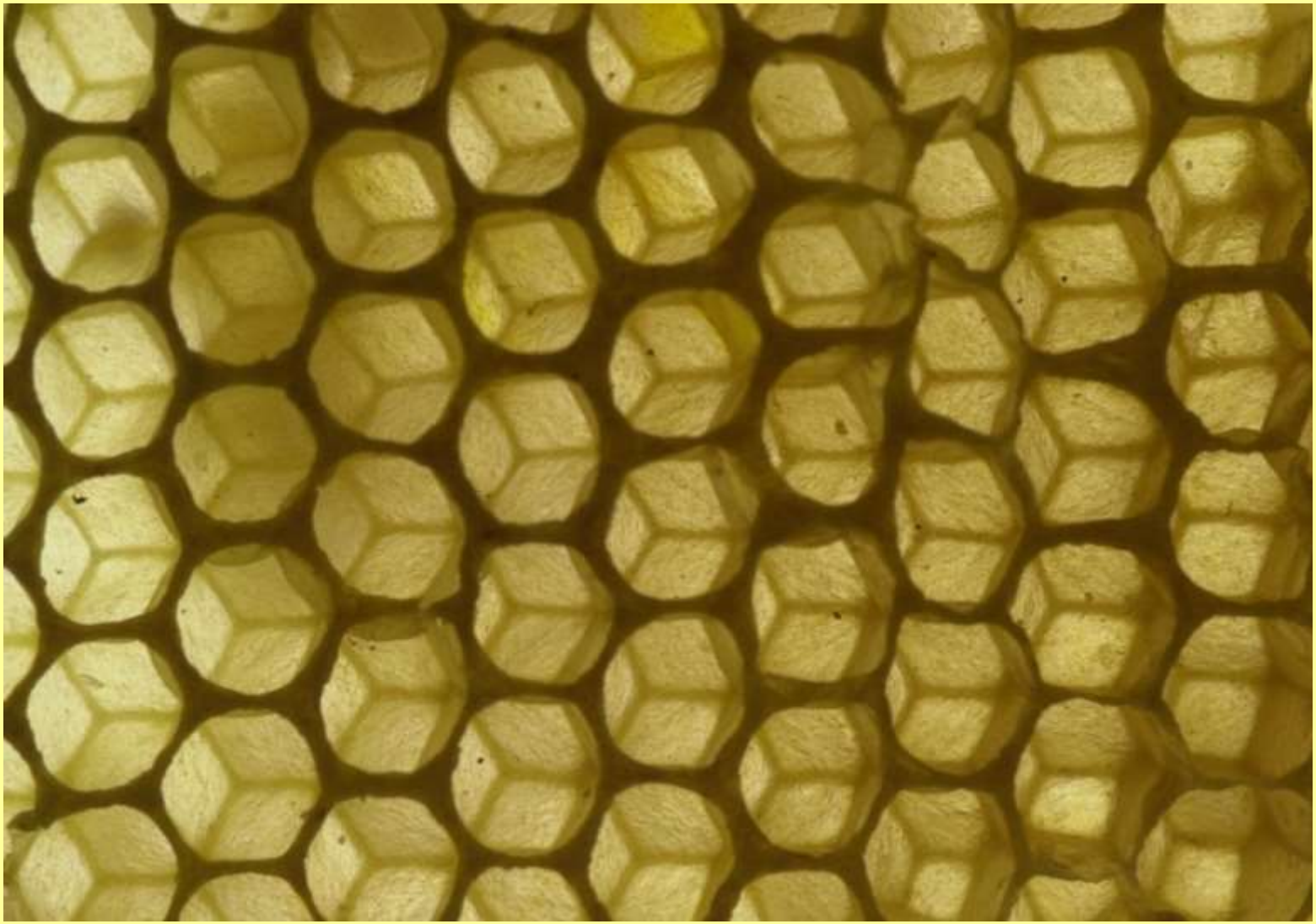
2. Oxidative Deamination of Glutamate

This final step converts glutamate back to a-ketoglutarate which enters the Kreb Cycle and is used in the production of stored chemical energy (NADH).

Glutamate + NAD^+ $\xrightarrow{\textit{Glutamate dehydrogenase}}$ *a*-ketoglutarate + NADH + NH_3

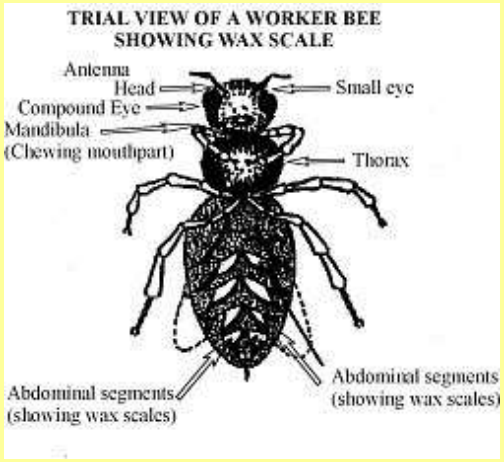
The Chemistry Of Beeswax

Honeybee Comb



The Chemistry of Beeswax

Beeswax is synthesized by 4 pairs of wax-secreting epidermal glands on the ventral side of the worker abdomens

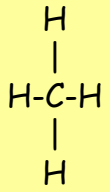


Beeswax is composed of a complex mixture of over 300 chemicals.

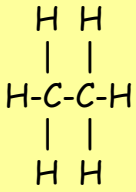
Long straight chain Hydrocarbons (14-20%),
Free Long straight change Fatty Acids (10-15%),
Free Long chain Fatty Alcohols (1-2%)
Long chain simple and complex Esters (65-75%)
(Monoesters, Diesters, Triesters, Acid Esters, Acid Polyesters,
Hydroxymonoesters, Hydroxypolyesters)
Other unknown Substances (4-8%)
(Aliphatic aldehydes, Ketones, beta-Diketones,
Triacylglycerols & more)

Hydrocarbons

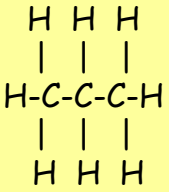
Examples of Simple Hydrocarbons, Very Flammable, Liquid



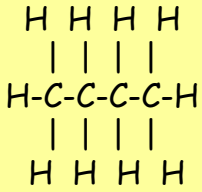
Methane CH4



Ethane CH3CH3



Propane CH3CH2CH3



Butane CH3(CH2)2CH3

Hydrocarbons in Beeswax

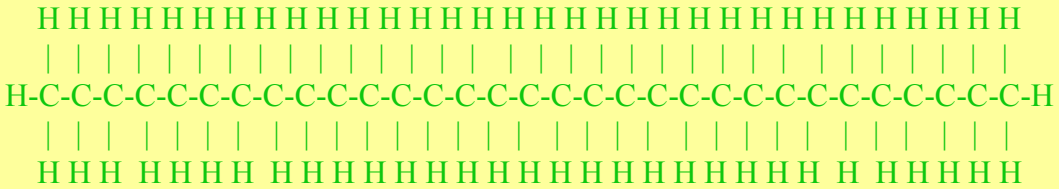
Hydrocarbons in Beeswax always contain an odd number of 23 to 35 carbons.

Long straight chain Hydrocarbons

14-20%of Beeswax

n-Hentriacontane, CH3(CH2)29CH3

8-9% of Beeswax



Very Flammable, Solid
31 Carbons

n-Heptacontane, CH3(CH2)25CH3

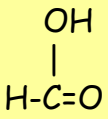
27 Carbons

n-Tritriacontane, CH3(CH2)31CH3

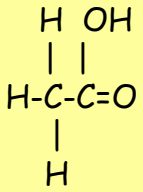
33 Carbons

Carboxylic Acids

Examples of Simple Carboxylic Acids, Very Strong Acids, Liquids, Flammable



Formic Acid, HCOOH



Acetic Acid, H3COOH

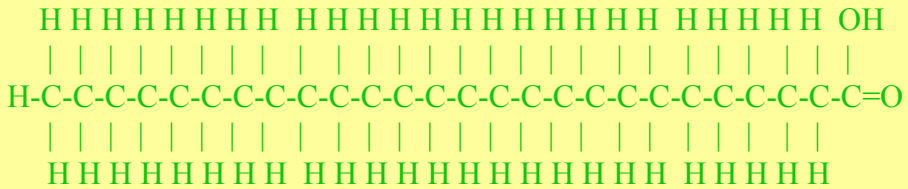
Free Long Straight Chain Fatty Acids in Beeswax

Fatty Acids in Beeswax always contain an even number of 16 to 36 carbons.

Thio-Claisen Reactions add 2 carbon atoms in the synthesis of Fatty Acids

Long straight chain Fatty Acids 10-15%of Beeswax

Cerotic Acid (Hexacosanoic Acid), CH3(CH2)24COOH



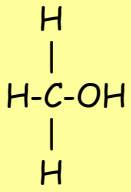
26 Carbons, Solid, Flammable, Weak Acid

Palmitic Acid, CH3(CH2)14COOH 16 Carbons

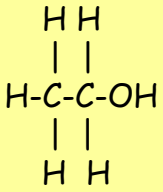
Melissic Acid, CH3(CH2)28COOH 30 Carbons

Alcohols

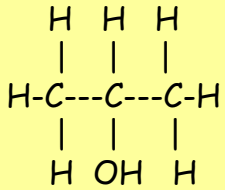
Examples of Simple Alcohols: Liquids, Flammable



Methanol, CH3OH



Ethanol, CH3CH2OH



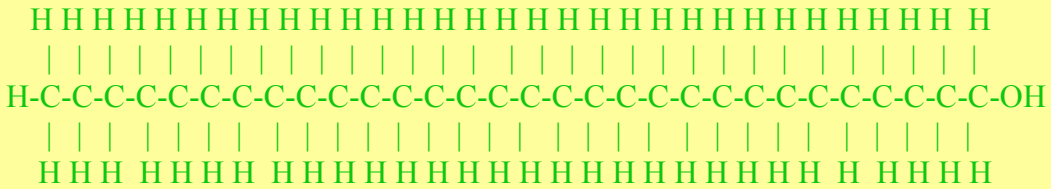
IsoPropanol or 2-Propanol CH3CHOHCH3

Free Long Straight Chain Fatty Alcohols in Beeswax

Fatty Alcohols in Beeswax always contain an even number of 14 to 40 carbons.

Long straight chain Fatty Alcohols 1-2%of Beeswax

Myricyl Alcohol or n-Triacontanol, $CH_3(CH_2)_{29}OH$

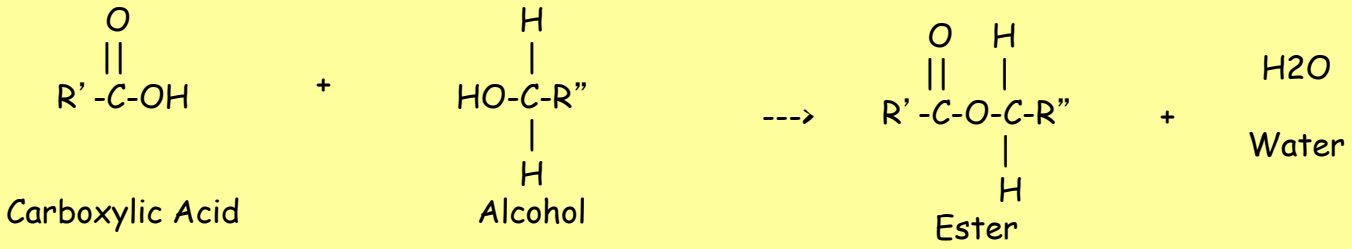


30 Carbons
Very Flammable, Solid

n-Tetracosanol, $CH_3(CH_2)_{23}OH$ 24 Carbons
n-Hexacosanol, $CH_3(CH_2)_{25}OH$ 26 Carbons
n-Tetracosanol, $CH_3(CH_2)_{27}OH$ 28 Carbons
n-Dotriacontanol, $CH_3(CH_2)_{31}OH$ 32 Carbons

Esters

Examples of Simple Ester: Liquids, Very Aromatic



Salicytic Acid + Methyl Alcohol --> Methyl Salicylate Wintermint extract
Acetic Acid + Isoamyl Alcohol --> Isoamyl acetate Banana extract
Butyric Acid + Ethyl Alcohol --> Ethyl Butyrate Pineapple extract

Esters in Beeswax

Long Fatty Acid + Long Fatty Alcohol --> Long Fatty Ester

Long Fatty Esters 65-75%of Beeswax

Esters in Beeswax always contain an even number of carbons.

Myricyl Palmitate, $CH_3(CH_2)_{14}COO(CH_2)_{29}CH_3$ ~23% of Beeswax

$CH_3(CH_2)_{14}COOH + HO(CH_2)_{29}CH_3 \rightarrow CH_3(CH_2)_{14}COO(CH_2)_{29}CH_3 + H_2O$

Palmitic Acid (16C) Myricyl Alcohol (30C) Myricyl Palmitate (46C) Monoester

Complex Esters: Diester, Triesters, Polyesters, Hydroxyesters, Acid Esters

Some physical Properties of Bees Wax

Melting Point: 61.7 to 62.8 C
Very Flammable
Moldable Solid
Water insoluble
7000 tons /year (world)

Questions?