Flavour formation in foods

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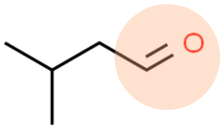
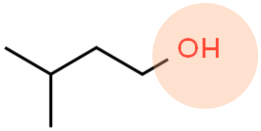
What are we learning today?

- How does flavour arise in foods?
- The chemistry of flavour formation
- How to identify the possible origin of a flavour compound

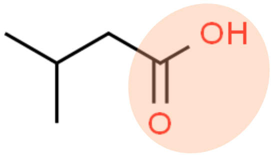
Some basic chemistry



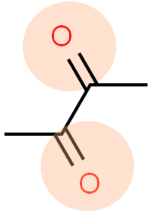
3-methylbutan**al**
aldehyde



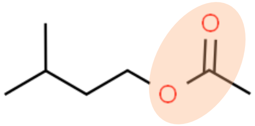
3-methyl-1-butan**ol**
alcohol



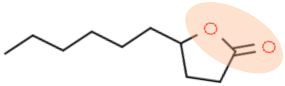
3-methylbutan**oic acid**
carboxylic acid



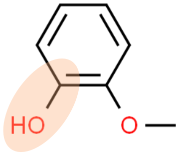
2,3-butanedi**one**
ketone



3-methylbut**yl** acet**ate**
ester
(an alcohol + a acid)



γ-deca**lactone**
lactone
(a cyclic ester)



2-methoxyphenol
phenol
("hydroxybenzene")

Origins of aroma compounds in foods

- **Biosynthesis**



- **Fermentation**



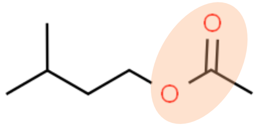
- **Thermal processing**


BIOSYNTHESIS

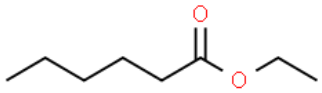
- Foods consumed raw: fruits, vegetables, salads, spices
- What is flavour for us are attractants or repellents for other animals
- Primary aroma compounds: Those formed via the plant's metabolism (growth, maturation, ripening).
- Secondary aroma compounds: Those formed after cell damage (chopping, peeling, squeezing, etc.).

Biosynthesis. Primary aroma compounds. Esters

- Primary aroma compounds: Those formed via the plant's metabolism (growth, maturation, ripening).
- Mostly esters and terpenes. Very important in fruit aroma

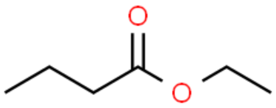


3-methylbutyl acetate
ester
(banana aroma)



Ethyl hexanoate

(pineapple, apple)



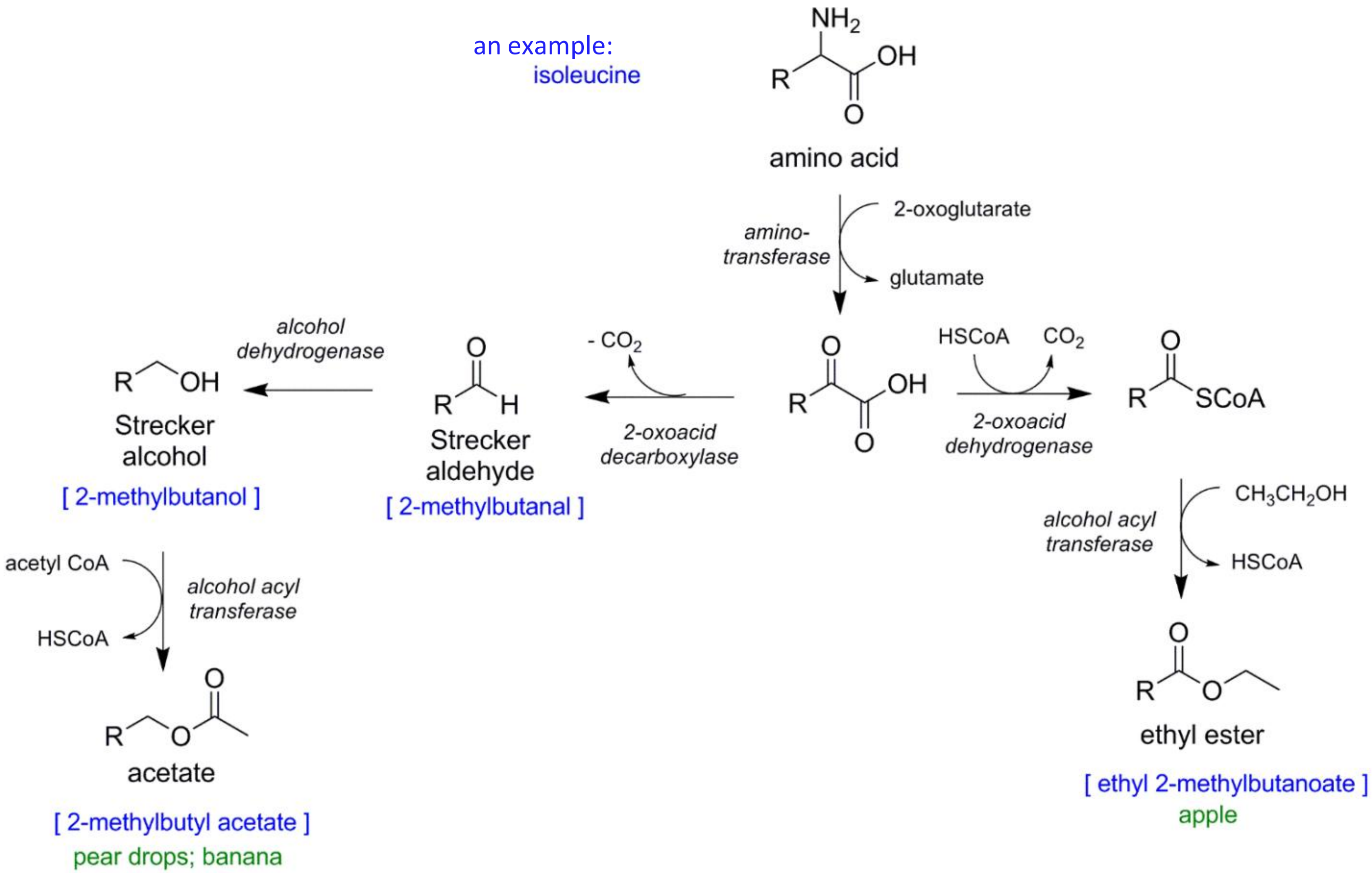
Ethyl butanoate

(strawberry, orange)

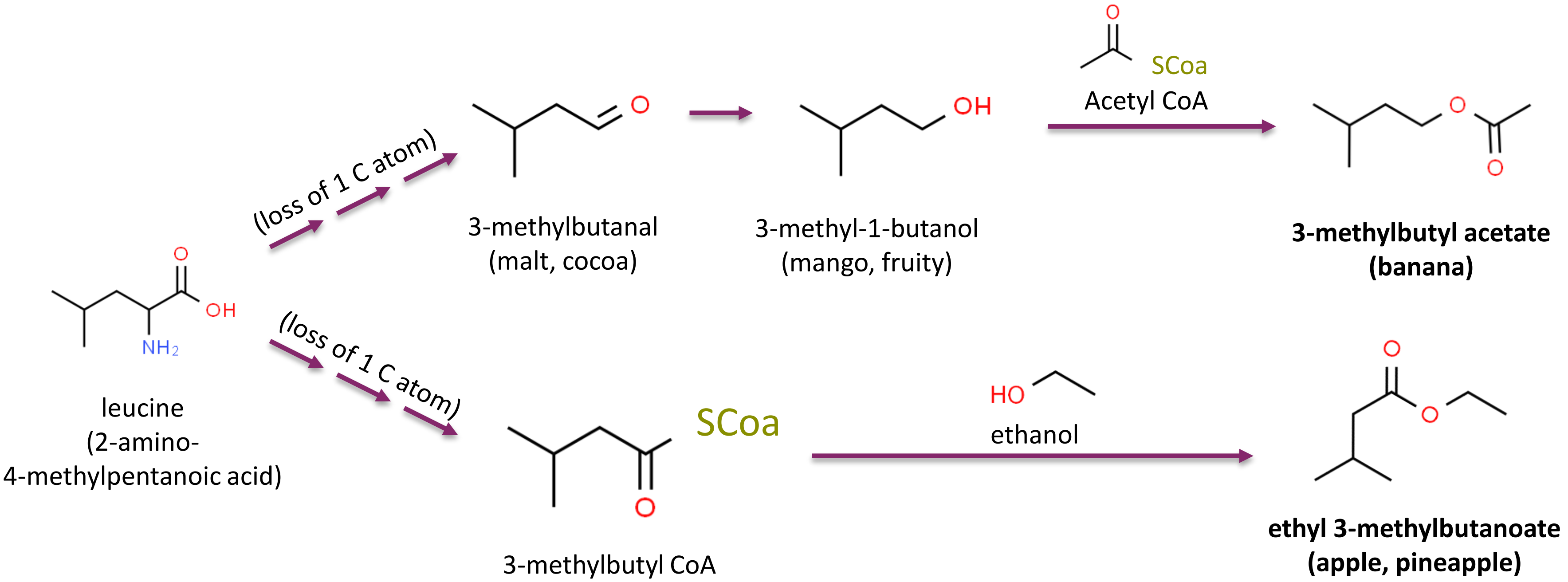
https://jameskennedymonash.wordpress.com/2013/12/13/infographic-table-of-esters-and-their-smells/

Biosynthesis of esters from amino acids

an example:
isoleucine



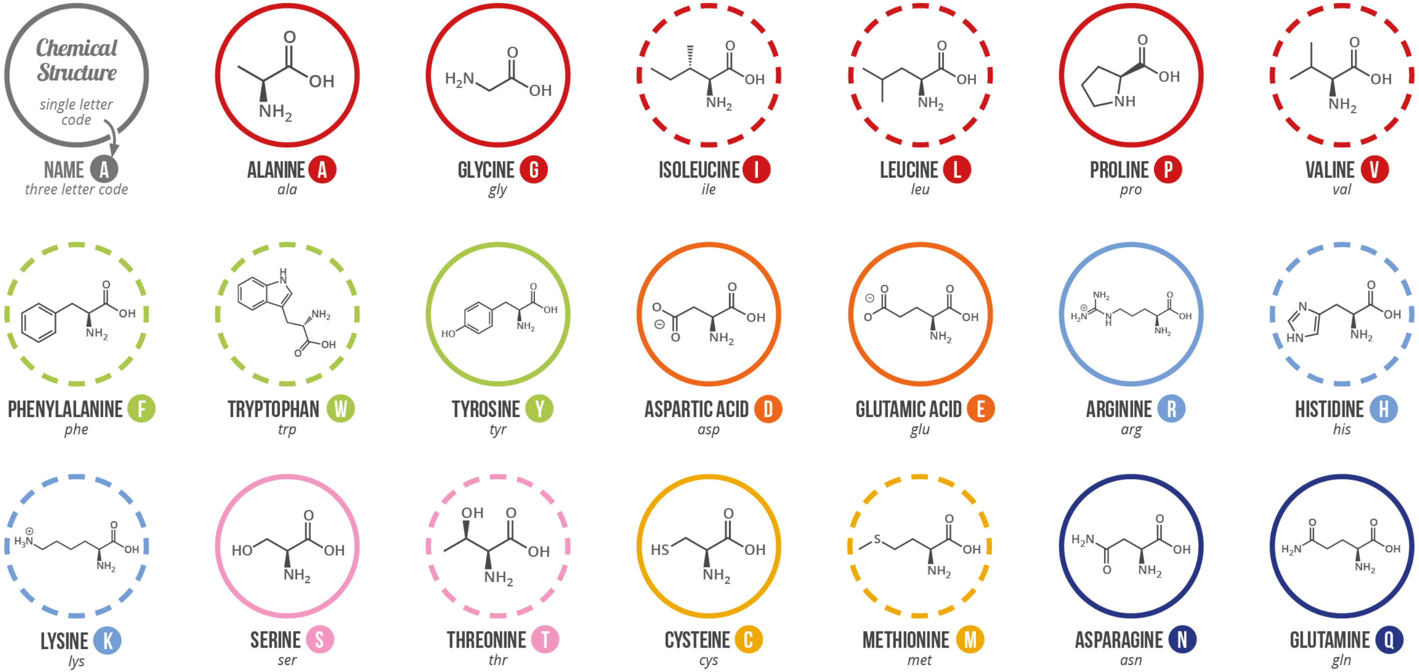
Biosynthesis of esters from amino acids



A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

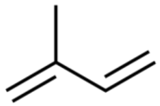
Chart Key: ALIPHATIC | AROMATIC | ACIDIC | BASIC | HYDROXYLIC | SULFUR-CONTAINING | AMIDIC | NON-ESSENTIAL | ESSENTIAL



Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

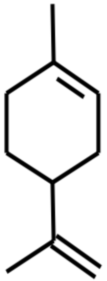
Terpenes

- Biosynthesised from isoprene (5 carbons) →

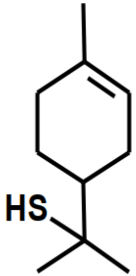


- Usually have 10 carbons (terpenes) or 15 carbons (sesquiterpenes)
- Called terpenoids when contain a heteroatom (other than C and H)
- Provide characteristic aroma to **herbs**, **spices**, **fruits** (specially citrus).

Some terpenes and terpenoids found in citrus fruits

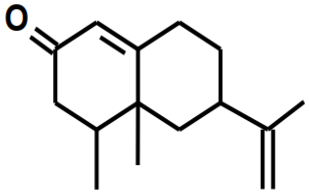


limonene
lemon

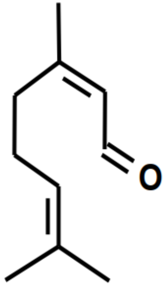


p-menthene-8-thiol
grapefruit

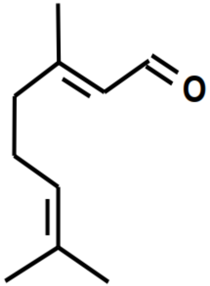
α-pinene
pine



nootkatone
grapefruit



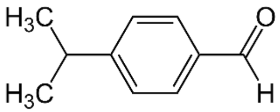
neral
lemon



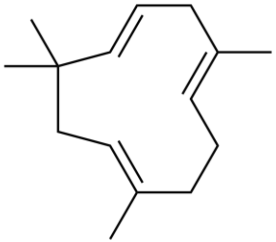
geranial
citrus, geranium

Other terpenes and terpenoids

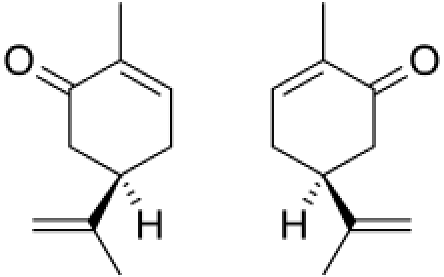
1,8-cineol (eucaliptol)
eucaliptus



Cuminaldehyde
(p-isopropylbenzaldehyde)
cumin



α-caryophyllene
(humulene)
hops

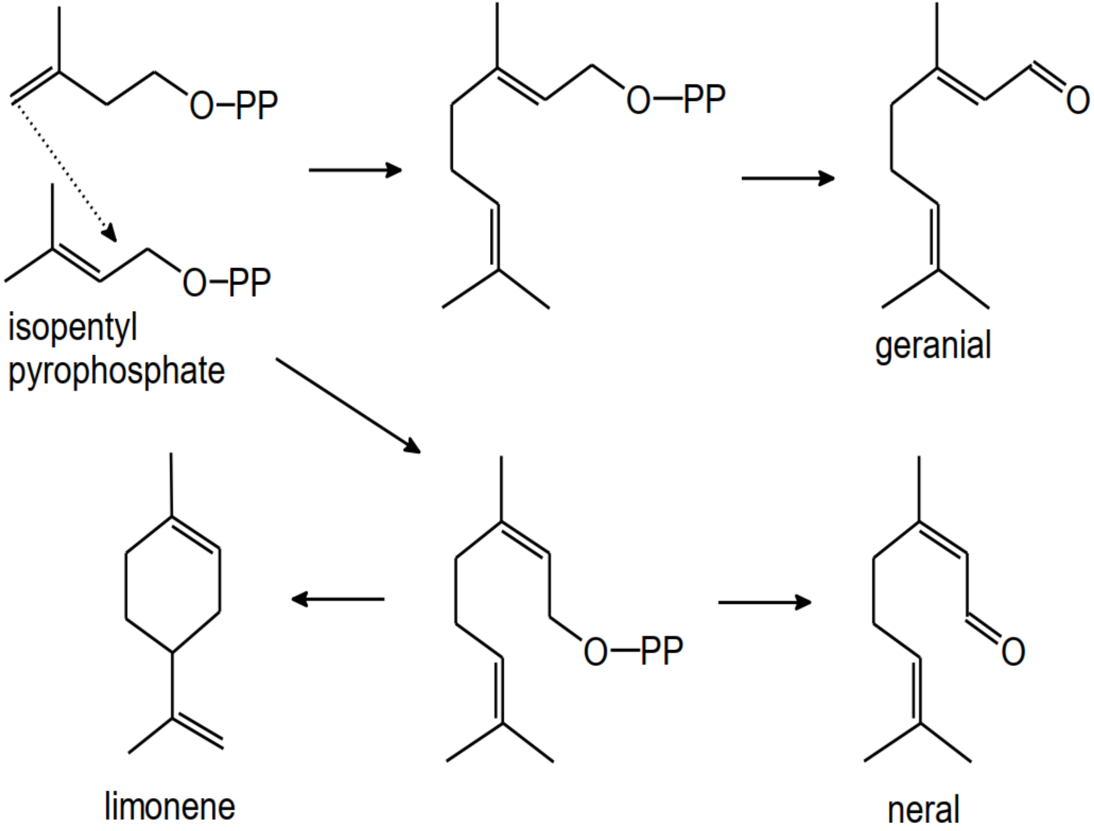


(R) (S)

Two stereoisomers, two different aroma qualities:

R-(-)-carvone spearmint
S-(+)-carvone caraway

Formation of terpenes in citrus fruits



- Isoprene units (5 carbons) are present as isopentyl pyrophosphate
- Two isopentyl units can bind to form a longer 10-carbon chain

Biosynthesis. Secondary aroma compounds

- <u>Secondary</u> aroma compounds: Those formed after cell damage.
- When cells are broken, enzymes get in touch with substrates and initiate the enzymatic reaction.
- Before cell disruption, enzymes and substrates were **physically separated**.
- Two examples: formation of aroma in *Allium* and formation of “green” odour aldehydes.

Allium species

Allium cepa
onion

Allium sativum
garlic

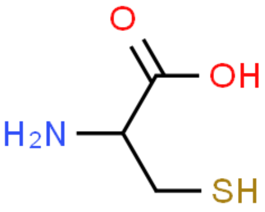
Allium cepa var. *aggregatum*
shallots

Allium schoenoprasum
chives

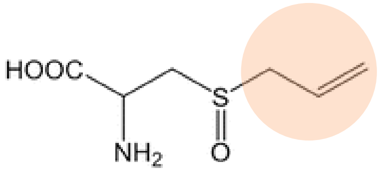
Allium ampeloprasum
leek

Sulfur compounds in *Allium*

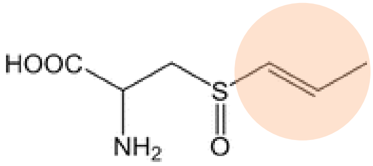
- Formed from alkyl or alkenyl (double bound) **sulfoxide derivatives** of the amino acid **cysteine**
- Enzyme: **alliinase**. Physically separated from the substrates; they get in contact after cell breakage.
- Alliin dominates in garlic (2 g/kg); isoalliin in onion (2.4 g/kg).



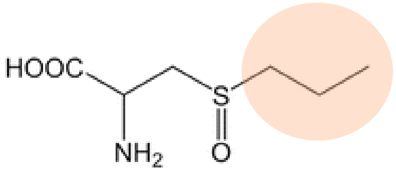
cysteine



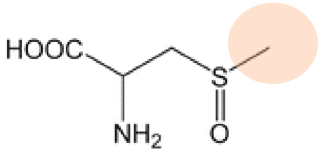
alliin



isoalliin



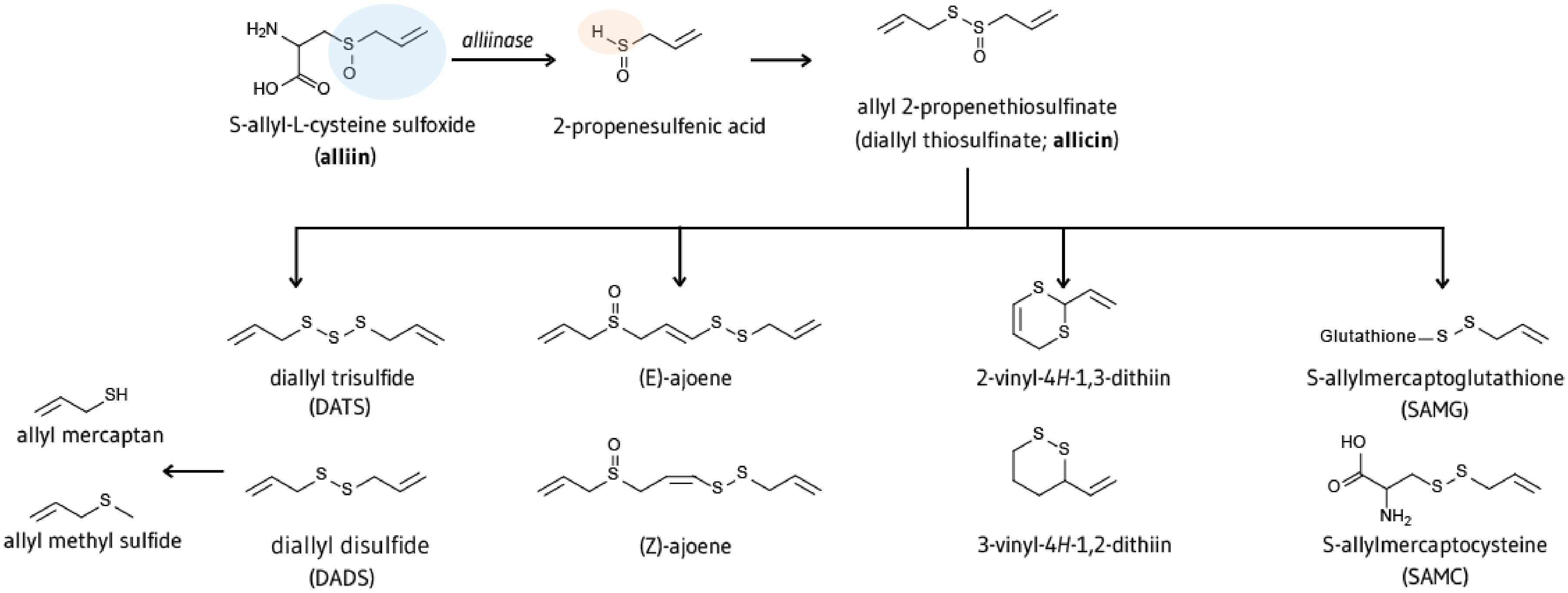
propiin

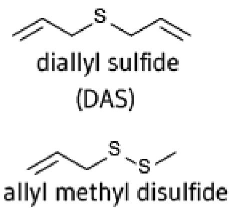


methiin

https://link.springer.com/chapter/10.1007/978-3-319-56526-2_5

Figure 2. Organosulfur Derivatives of Alliin in the Process of Garlic Product Preparation





Sulphide bonds (-S-) can break and rearrange easily, giving a large variety of compounds. Also form di- (-S-S-), tri- (-S-S-S-) and tetrasulphides (-S-S-S-S-). The longer, the more unstable.

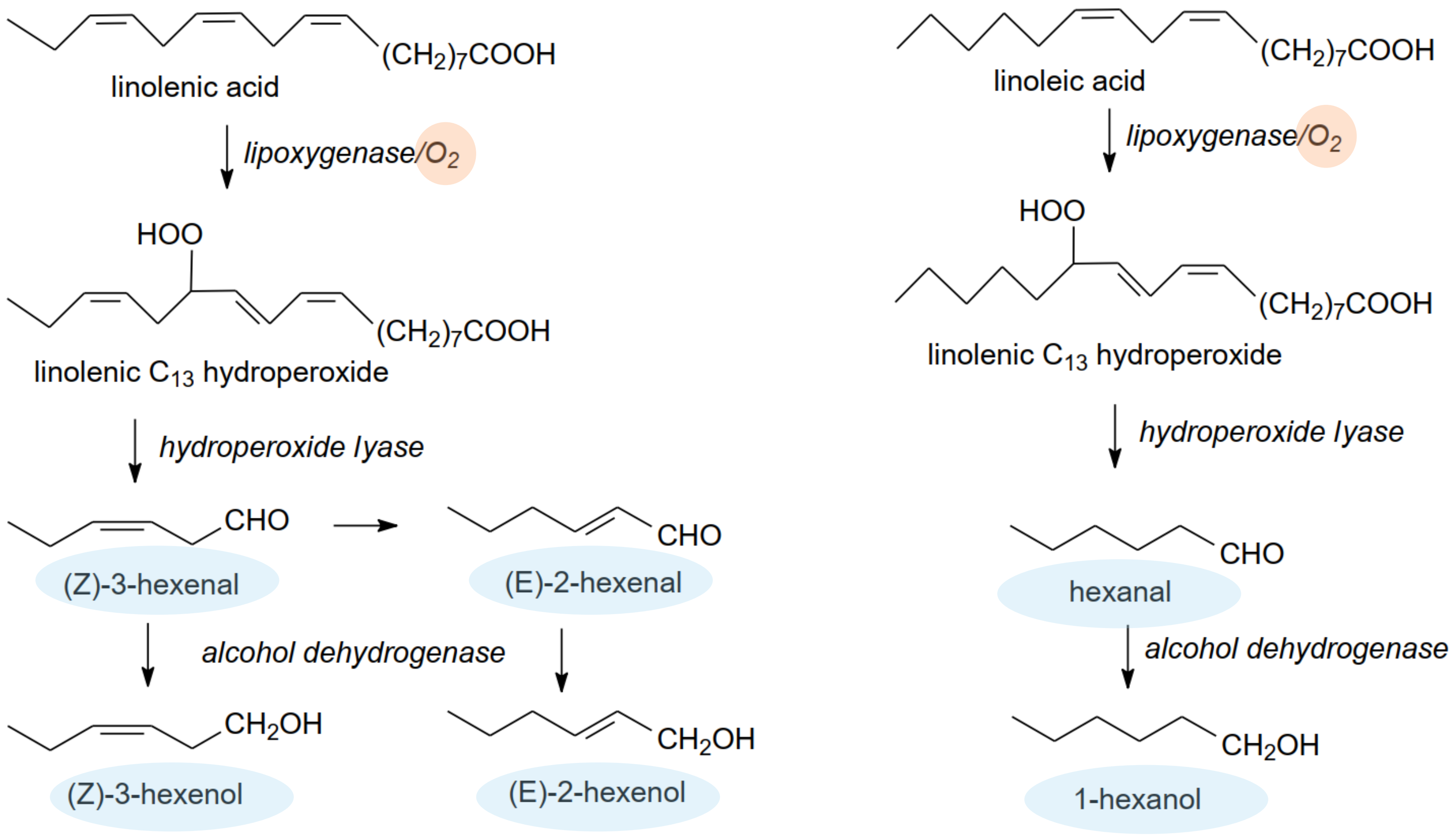
Sulphur aroma compounds

- Very potent aroma compounds. We can perceived them even at extremely low concentrations.
- They have a very characteristic aroma: **garlic**, **onion**, but also **faecal**.
- Unstable compounds. They decompose easily.
- Can form sulphides, disulphide, trisulphides…

Formation of “green” aromas. Enzymatic lipid oxidation

- Formed by the oxidation of lipids caused by **lipoxygenase** activity. It requires the presence of **oxygen**!
- The result is **linear** aldehydes and alcohols with usually **6 or 9 carbons**, with very potent “green” or “fresh cut grass” aroma.
- Very potent; they can be perceived even in foods with very low fat content.
- Key aroma compounds in legumes (green peas, soya, etc.), cucumber, herbs, vegetables in general, oils (in excess, they are perceived as rancid*).

*Rancidification of oils can occur by other chemical processes, not only lipoxygenase activity, although the aroma compounds formed are similar.



(CH2)7COOH
linolenic acid
lipoxygenase/O2
HOO
(CH2)7COOH
linolenic C13 hydroperoxide
hydroperoxide lyase
CHO
(Z)-3-hexenal
CHO
(E)-2-hexenal
alcohol dehydrogenase
CH2OH
(Z)-3-hexenol
CH2OH
(E)-2-hexenol
(CH2)7COOH
linoleic acid
lipoxygenase/O2
HOO
(CH2)7COOH
linolenic C13 hydroperoxide
hydroperoxide lyase
CHO
hexanal
alcohol dehydrogenase
CH2OH
1-hexanol

FERMENTATION

- Fermentative processes not only help degrade proteins, carbohydrates and other macromolecules to make them more easily digestible.
- Lots of aroma compounds are formed in **enzymatic** reactions.
- From **amino acids**, we obtain mostly alcohols, esters and organic acids (Similar to the aroma compounds found in fruits by biosynthesis).

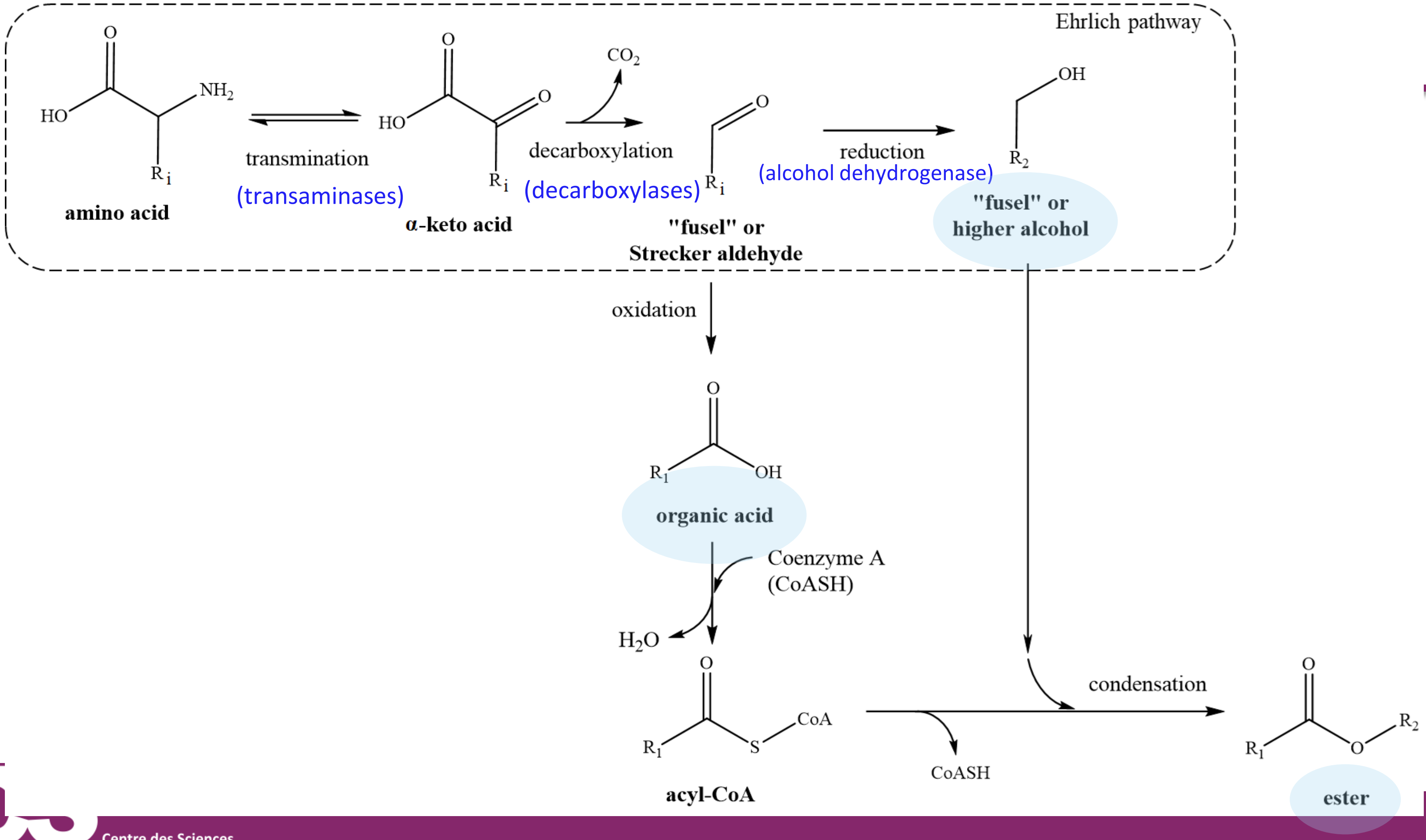
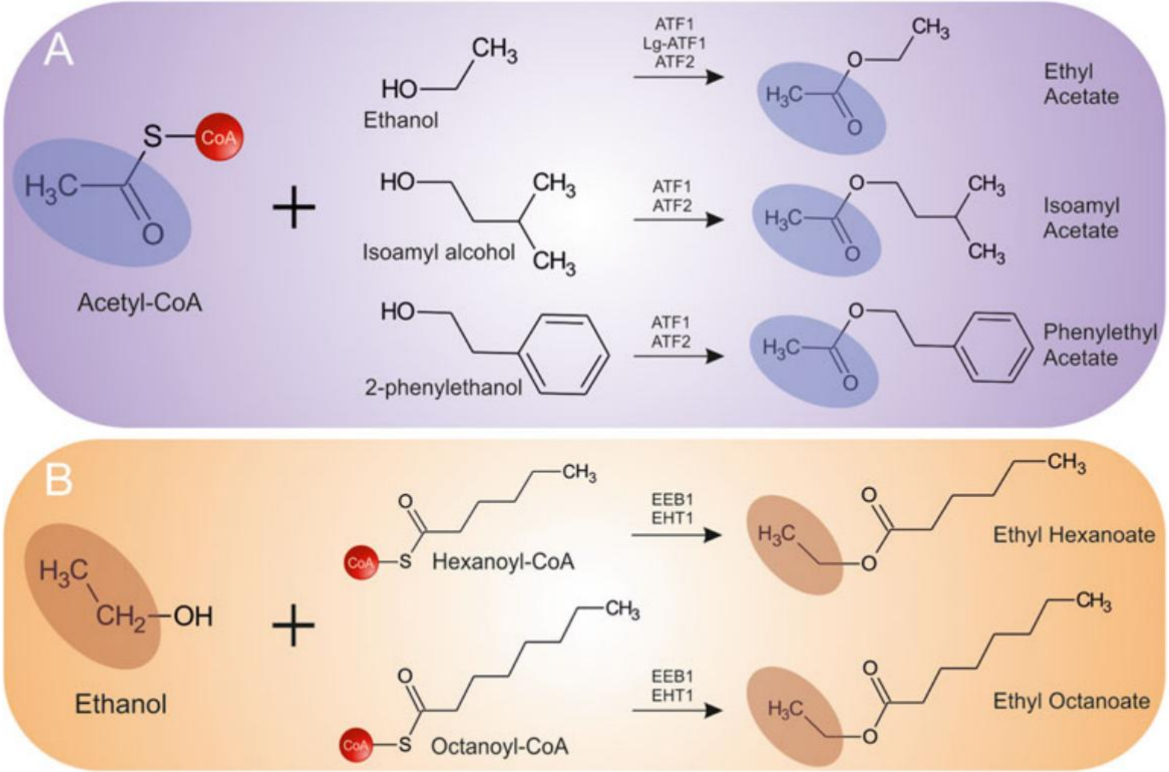

Ehrlich pathway
O
HO
NH2
Ri
amino acid
transmination
(transaminases)
O
HO
O
Ri
α-keto acid
CO2
decarboxylation
(decarboxylases)
O
Ri
"fusel" or
Strecker aldehyde
reduction
(alcohol dehydrogenase)
OH
R2
"fusel" or
higher alcohol
oxidation
O
R1
OH
organic acid
Coenzyme A
(CoASH)
H2O
O
R1
S
CoA
acyl-CoA
CoASH
condensation
O
R1
O
R2
ester

Fig. 3 A scheme of the chemical reactions involving the biosynthesis of acetate esters (**a**) and medium-chain fatty acid ethyl esters (**b**). The main genes involved in each reaction are presented above the reaction arrows



Esters and alcohols in beer

- Beer fermentation by yeast produces a huge variety of fruity alcohols and esters that give their characteristic aroma.
- Esters are predominant in beer and are responsible for the fruity flavour.
- These compounds are also present in other fermented beverages, such as rum, whiskey, wine, sake, etc.
- Also present in other fermented foods like cheese and bread.

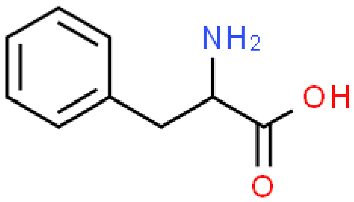
Table 1 Threshold values of most important esters and higher alcohols present in lager beer (Engan 1974; Meilgaard 1975b; Engan 1981)

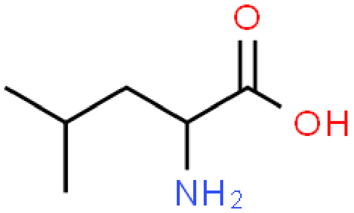
Compound	Threshold ($mg\ L^{-1}$)	Concentration range ($mg\ L^{-1}$)	Aroma impression
Acetate esters			
Ethyl acetate	25–30	8–32	Fruity, solvent
Isoamyl acetate	1.2–2	0.3–3.8	Banana
Phenylethyl acetate	0.2–3.8	0.1–0.73	Roses, honey
MCFA ethyl esters			
Ethyl hexanoate	0.2–0.23	0.05–0.21	Apple, fruity
Ethyl octanoate	0.9–1.0	0.04–0.53	Apple, aniseed
Higher alcohols			
n-Propanol	600	4–17	Alcohol, sweet
Isobutanol	100	4–57	Solvent
Isoamyl alcohol	50–65	25–123	Alcoholic, banana
Amyl alcohol	50–70	7–34	Alcoholic, solvent
2-Phenylethanol	40	5–102	Roses

Pires et al. 2014
DOI: 10.1007/s00253-013-5470-0

Exercise 1.

What aroma compounds can we get from phenylalanine and leucine (together in the same system) by fermentation? Take into account the presence of ethanol and acetyl-SCoA





THERMAL PROCESSING

Formation of a huge variety of aroma, taste and colour compounds.

- **Caramelisation**
- **Maillard reaction**



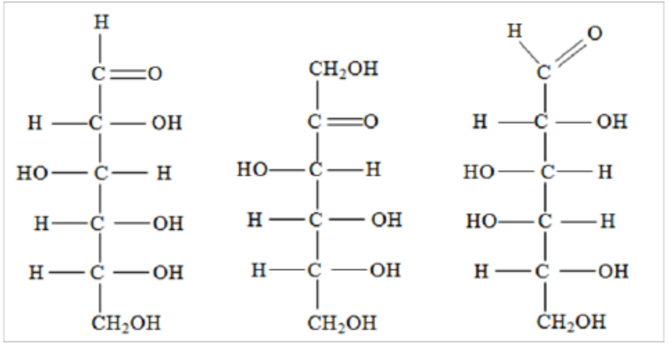
Caramelisation

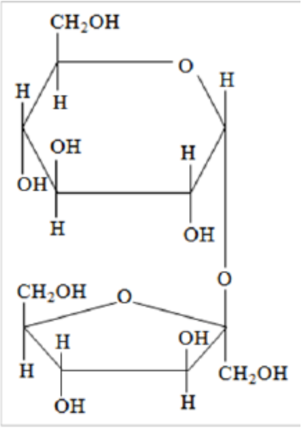
- Occurs in food products with a **high sugar** content.
- Requires **high temperature**, above 120 °C.
- It involves the thermal degradation of sugars, usually **mono-** but also **di-** and **trisaccharides**.
- Since sugars contain only C, H and O, the compounds formed thereafter will only contain these atoms.
- Linear but also cyclic compounds are formed.

Caramelisation

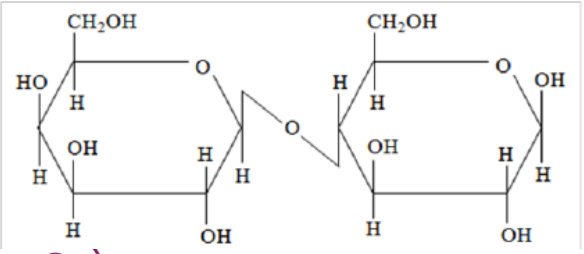
Some cyclic aroma compounds:

Some sugar molecules:





Sucrose



Lactose

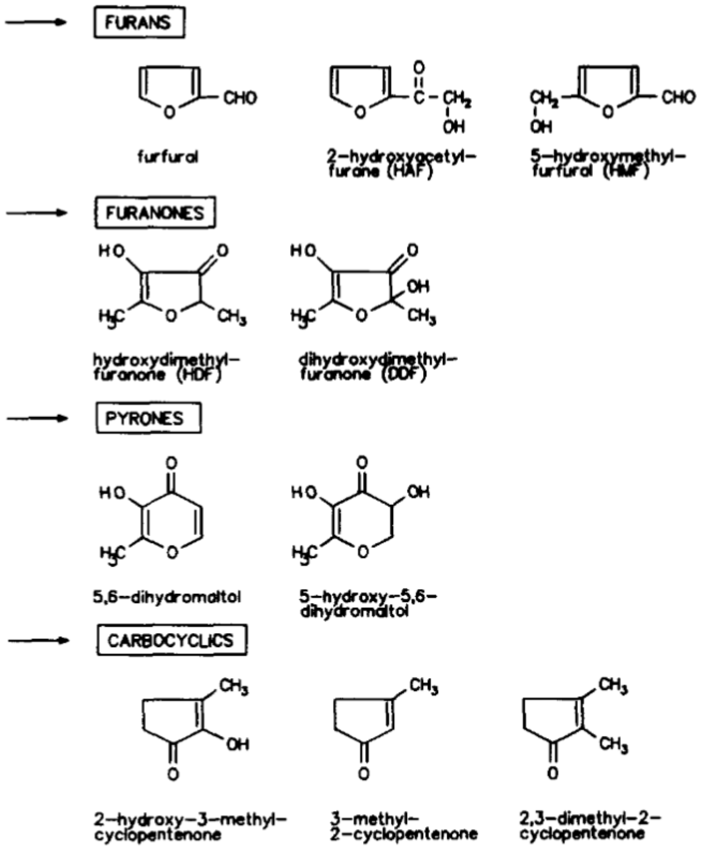


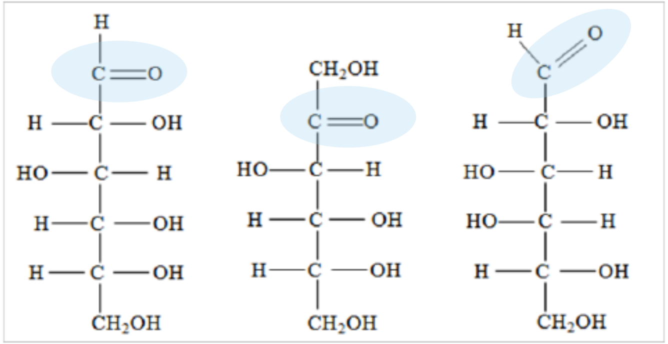
Fig. 2. Typical caramel aromatics.

Maillard reaction

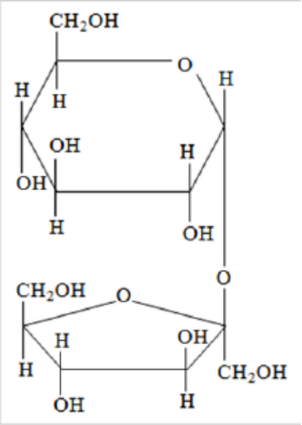
REDUCING SUGAR + AMINO COMPOUND

in food, usually an **amino acid**

- All monosaccharides
- **However**, not all other bigger sugars are reducing sugars

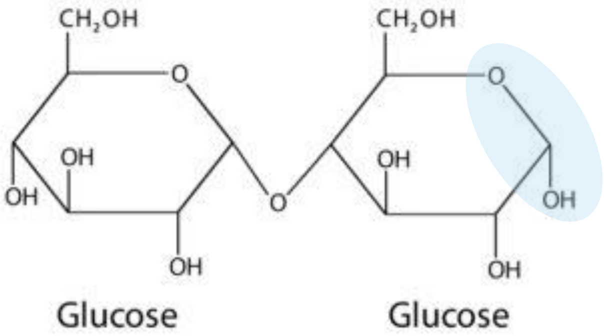


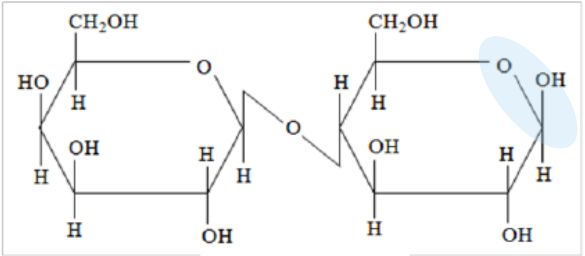
D-Glucose D-Fructose D-Galactose



Sucrose

Maltose



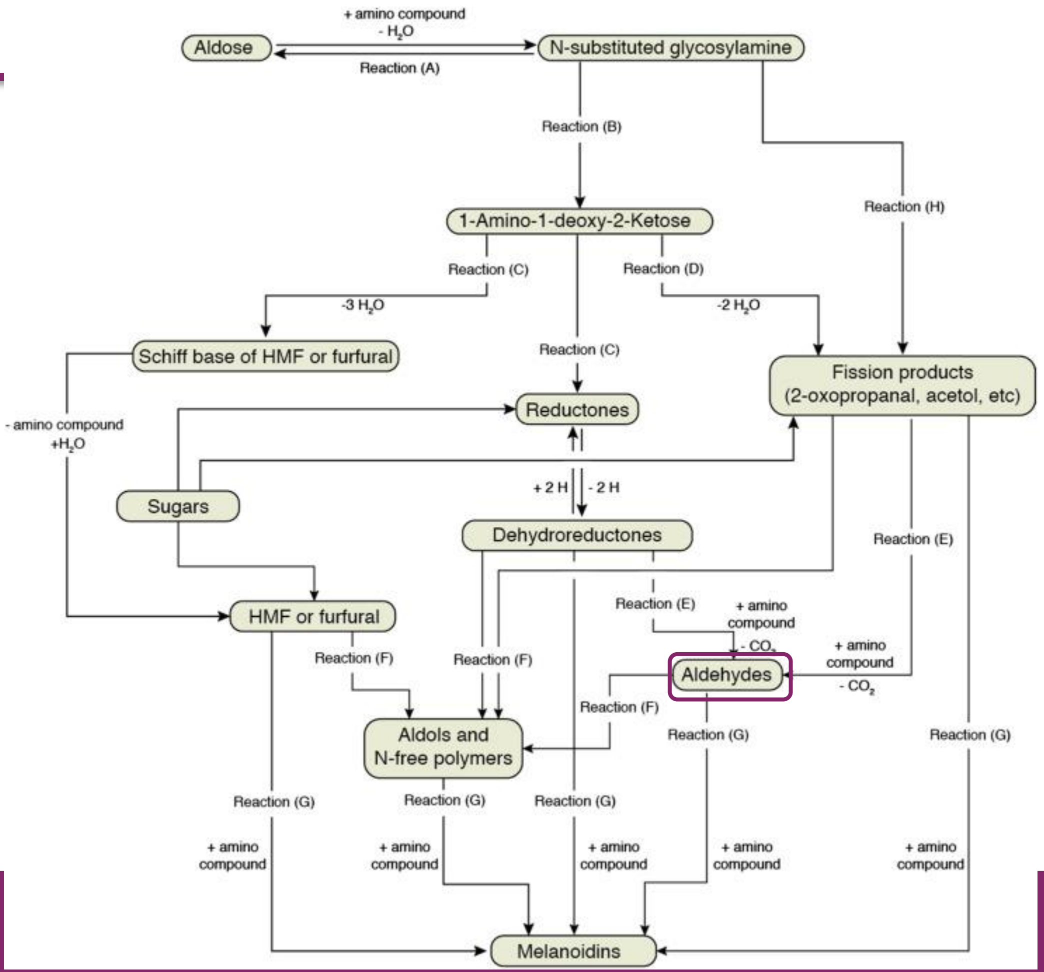


Lactose

Sucrose is **NOT** a reducing sugar.
It cannot initiate the Maillard reaction.

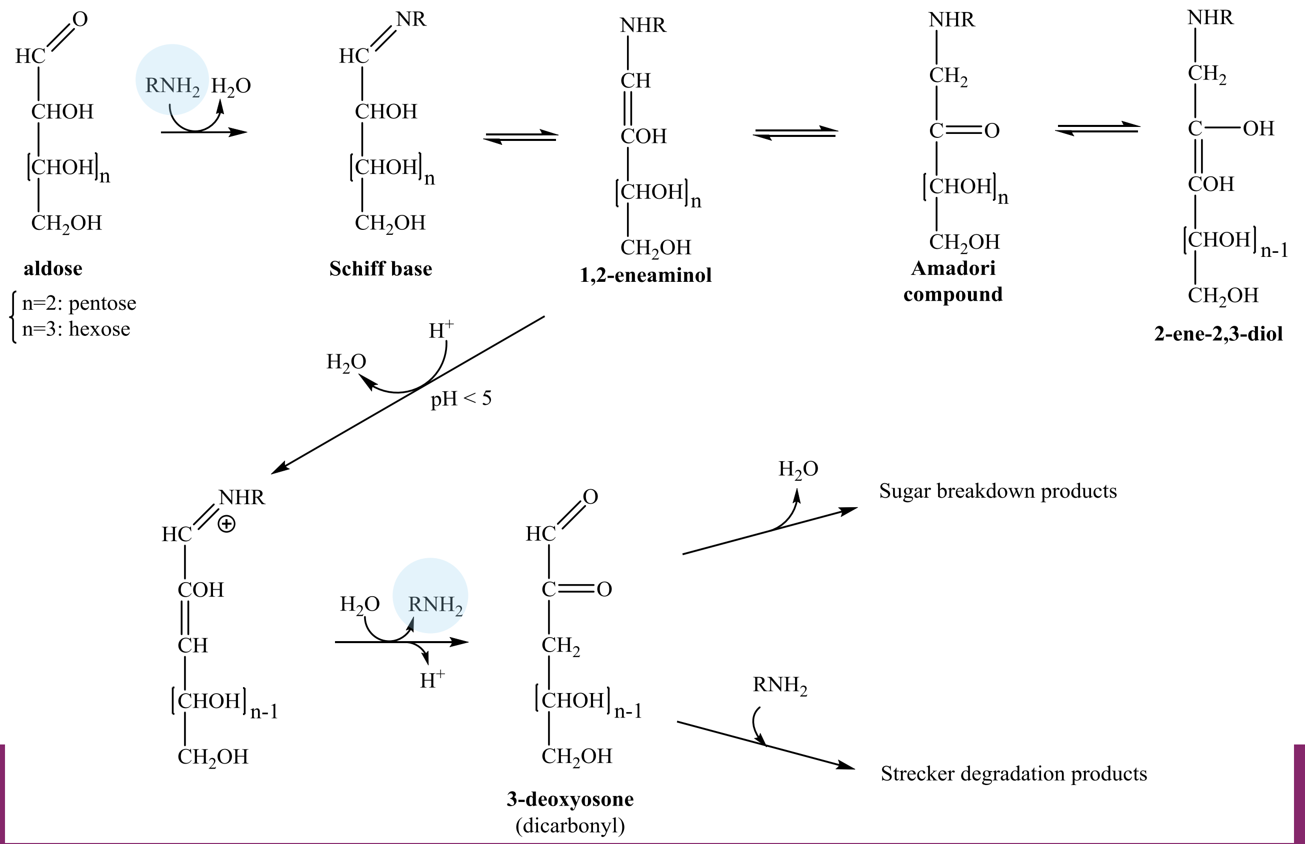
Maillard reaction

- It is a very complex network of parallel and consecutive reactions.
- The final product, **melanoidins**, are a pool of heterogenous compounds with yellow or brown colour (Maillard reaction → Non-enzymatic browning)



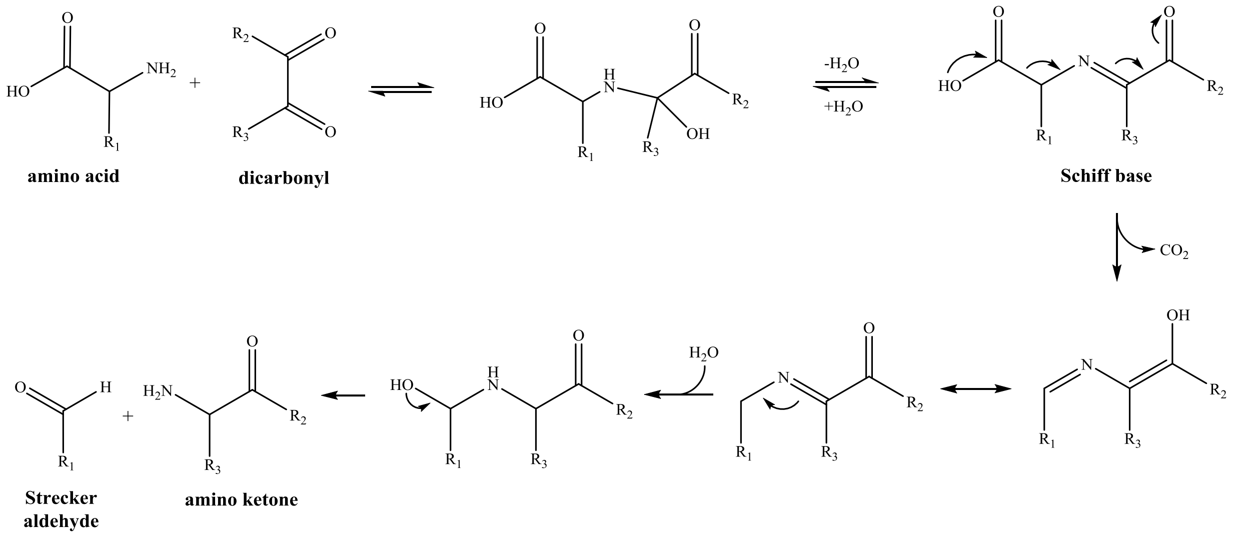
Maillard reaction: Early stages

- The amino compound is released and a very reactive dicarbonyl is formed.
- This dicarbonyls reacts further with other compounds like amino compounds or it breaks down into other products.



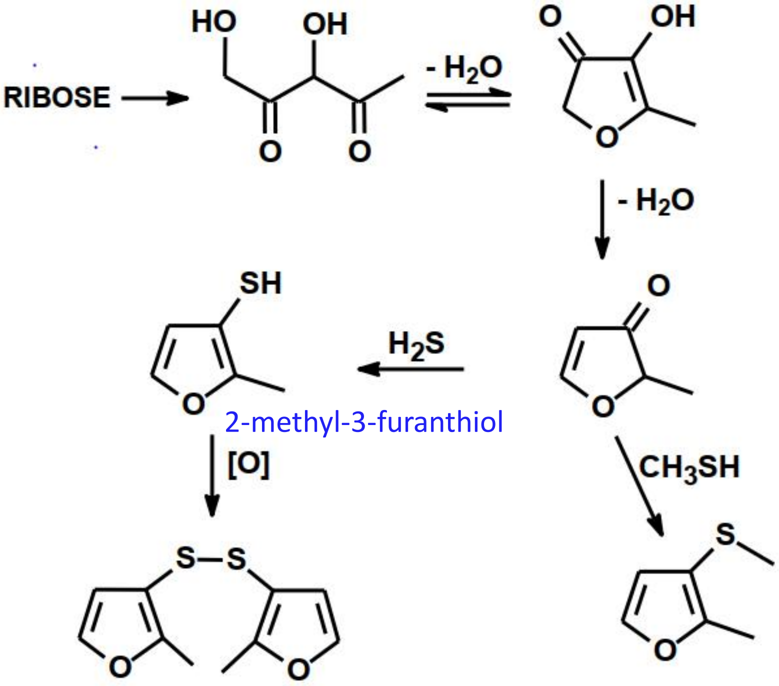
Strecker degradation: Formation of aldehydes

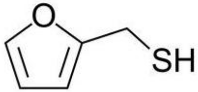
- When the dicarbonyls reacts with an amino acid, an aldehyde is formed via the Strecker degradation.
- This aldehyde will have one carbon less than the amino acid of origin.



Sulphur compounds from cysteine

- Formation of character impact **meaty** compounds
- Ribose is a monosaccharide present in meat. Cysteine is an amino acid containing sulphur.
- These compounds are also key in **coffee aroma**.
- Vegetables and fruits do not contain cysteine and ribose, that is why they do not produce meaty aromas.





furfurylthiol
another important aroma compound in meat and coffee

- Although the Maillard reaction is usually associated with thermal processes, it is a **spontaneous reaction**.
- It can happen **very slowly** at room temperature. Higher temperatures increase the reaction rate.
- Ripened cheeses, aged Port wine

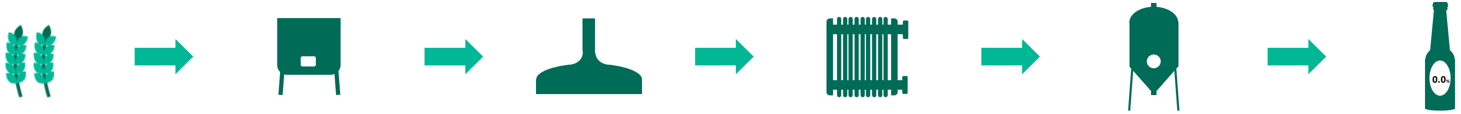
How can we guess the origin of an aroma compound?

- **Ingredients**
  - **Composition. Sugar? Fats? Proteins?**
- **Processing**
  - **Is there a thermal process involved?**
  - **Is the product fermented?**
  - **Both?**
- **Nature of the aroma molecule**
  - **Functional groups. Aldehyde? Ester? Terpene? Cyclic compound?**
  - **Could it be formed from an amino acid?**

Summary

- **Biosynthesis**
  - Primary: Esters, terpenes, terpenoids
  - Secondary: Linear aldehydes (saturated and unsaturated, usually C6 and C9), sulphides in *Allium*
- **Fermentation**
  - Alcohols, organic acids, esters from amino acids
- **Thermal processing**
  - Caramelisation: a variety of linear, branched and cyclic compounds (C,H,O)
  - Maillard reaction: Aldehydes from amino acids, heterocyclic compounds (C,H,O,**N**,**S**), ketones, sulphur compounds…

Example: Aroma compounds in beer



Malting
Barley grains are rehydrated, germinated and kilned

Mashing
Water is added to malt and starch is hydrolysed

Wort boiling
Spent grains are removed, hops are added and the wort is boiled

Centrifuging and cooling
Hops are separated from the wort and this is cooled down

Fermentation
Wort is fermented by yeast

Post-treatments
Maturation, flavour addition and bottling, among others

Could you identify where aroma compounds can be potentially formed?

Table 2. Most Odor-Active Volatiles in Bavarian Wheat Beer A (FD Factor ≥ 16)

no.[b]	odorant[c]	odor quality[d]	RI[a] on FFAP	RI[a] on SE-54	FD factor[e]
1	methylpropanol	malty	1100	<700	1024
2	3-methylbutyl acetate	fruity, banana-like	1130	881	512
3	3-methylbutanol	malty	1225	758	2048
4	ethyl hexanoate	fruity	1246	1007	32
5	1-octen-3-one[f]	mushroom-like	1300	969	16
6	2-acetyl-1-pyrroline[f]	roasty, popcorn-like	1317	923	64
7	ethyl octanoate	fruity	1430	1168	64
8	acetic acid[g]	sour, pungent	1435	<700	2048
9	3-(methylthio)propanal	cooked potato-like	1452	915	1024
10	linalool	flowery, citrus-like	1533	1107	32
11	methylpropanoic acid[g]	sweaty	1559	823	16
12	butanoic acid[g]	sweaty	1621	862	512
13	phenylacetaldehyde	honey-like	1662	1048	64
14	2- and 3-methylbutanoic acid[g]	sweaty	1663	881	2048
15	3-(methylthio)propanol	cooked potato-like	1710	92	2048
16	unknown[g]	earthy	1732	nd[h]	16
17	unknown	roasty	1747	1168	16
18a	2-phenylethyl acetate	flowery	1816	1260	1024
18b	(*E*)-*β*-damascenone	cooked apple-like	1816	1379	512
19	hexanoic acid[g]	sweaty, goat-like	1842	1029	16
20	2-methoxyphenol	smoky, woody	1863	1093	1024
21	unknown	fruity	1884	1354	16
22	2-phenylethanol	flowery	1922	1125	4096
23	unknown[g]	earthy, fatty	2000	nd	256
24	4-ethyl-2-methoxyphenol	smoky	2012	1154	256
25a	*γ*-nonalactone	coconut-like	2029	1367	128
25b	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[g]	caramel-like	2029	1075	32
26	octanoic acid[g]	sweaty, goat-like	2056	1283	16
27	unknown[g]	caramel-like	2127	1032	16
28	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[g]	seasoning-like, spicy	2206	1119	512
29	2-methoxy-4-vinylphenol	clove-like	2212	1321	4096
30	2-aminoacetophenone	foxy	2235	1354	128
31	unknown	metallic, geranium-like	2276	1491	256
32	4-vinylphenol	smoky, leather-like	2393	1228	512
33	phenylacetic acid[g]	honey-like	2520	1262	32
34	vanillin[g]	vanilla-like	2573	1392	256

[a]RI, linear retention index. [b]Numbering refers to Figures 1 and 2. [c]Compound was identified by comparison with reference substance on the basis of the following criteria: retention indeces (RI) on the capillaries detailed in the table, mass spectra obtained by MS-EI and MS-CI, odor quality as well as odor intensity perceived at the sniffing port. [d]Odor quality perceived at the sniffing port. [e]FD, flavor dilution factor. [f]MS signals were too weak for an unequivocal interpretation. Compound was identified on the basis of the remaining criteria given in footnote *b*. [g]Compound was identified in the fraction of the acidic volatiles. [h]nd, not determined.

Langos et al. 2013
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Exercise 2. Origin of these aroma compounds?

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Alcohols

1.	methylpropanol
3.	3-methylbutanol
15.	3-(methylthio)-propanol
22.	2-phenylethanol

Aldehydes

9.	3-(methylthio)-propanal
13.	phenylacetaldehyde

Organic acids

8.	acetic acid
11.	methylpropanoic acid
12.	butanoic acid
14a.	2-methylbutanoic acid
14b.	3-methylbutanoic acid
19.	hexanoic acid
26.	octanoic acid
33.	phenylacetic acid

Esters

2.	3-methylbutyl acetate
4.	ethyl hexanoate
7.	ethyl octanoate
18a.	2-phenylethyl acetate

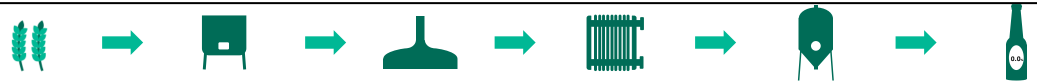
Terpenes

10.	linalool

Cyclic compounds

6.	2-acetylpyrroline
25b.	4-hydroxy-2,5-dimethyl-3(*2H*)-furanone
28.	3-hydroxy-4,5-dimethyl-2(*5H*)-furanone

Origin of these aroma compounds?



Malting	Mashing	Wort boiling	Centrifuging and cooling	Fermentation	Post-treatments
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1.	methylpropanol
3.	3-methylbutanol
15.	3-(methylthio)-propanol
22.	2-phenylethanol

Aldehydes	**THERMAL (Maillard)**
9.	3-(methylthio)-propanal
13.	phenylacetaldehyde

Organic acids	**FERMENTATION**
8.	acetic acid
11.	methylpropanoic acid
12.	butanoic acid
14a.	2-methylbutanoic acid
14b.	3-methylbutanoic acid
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26.	octanoic acid
33.	phenylacetic acid

Esters	**FERMENTATION**
2.	3-methylbutyl acetate
4.	ethyl hexanoate
7.	ethyl octanoate
18a.	2-phenylethyl acetate

Terpenes	**BIOSYNTHESIS**
10.	linalool

Cyclic compounds		**THERMAL**
6.	2-acetylpyrroline	**Maillard**
25b.	4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	**Maillard or caramelisation**
28.	3-hydroxy-4,5-dimethyl-2(*5H*)-furanone	**Maillard or caramelisation**

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Malting → **THERMAL PROCESSING (kilning)**

Wort boiling → **THERMAL PROCESSING (boiling)**

Wort boiling → **BIOSYNTHESIS (hops)**

Fermentation → **FERMENTATION (yeast)**