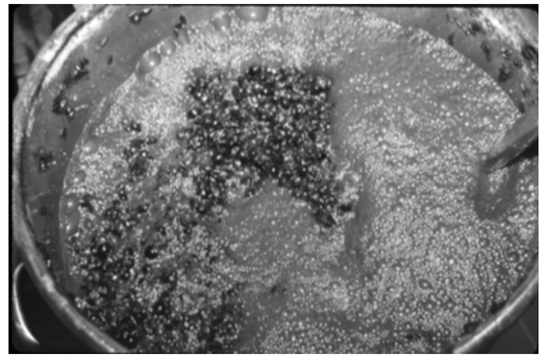
Vineyard Site
Cultivar Selection
Vineyard Management
Grape Maturity & Harvest
Juice Extraction

Alcoholic Fermentation

Malolactic Fermentation
Wine Aging (Storage)

Alcoholic Fermentation - fermentation pathway



Alcoholic Fermentation (acetaldehyde final electron acceptor)

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP + heat$
glucose ethanol

Respiration (complete oxidation)

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP + heat$

Yeast cell - membrane structures

Figure 8 : Yeast molecular structure.
Cw, Cell wall - Bs, Bud scar - P, Plasmalemme - I, Plasma membrane invagination - V, Vacuole - PG, Phosphate granule - LG, Lipid granule - Sp, Spherosome - M, Mitochondria - N, Nucleus - NE, Nuclear envelop - ER, Endoplasmic reticulum - G, Golgi - E, Endosome - B, Bud - Sv, Secretory vesicles.

Yeast cell wall

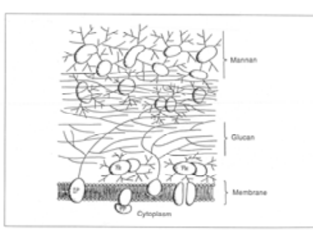
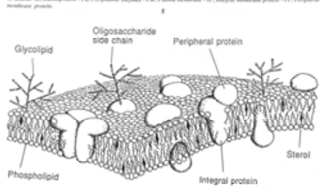


Figure 3 :Cell Wall Structure according to Schekman and Novick (Cold Spring Harbor Laboratory, 1987).
G, Glucan - M, Mannoprotein - PE, Periplasmic enzymes - PM, Plasma membrane - IP, Integral membrane protein - PP, Peripheral membrane protein.

Yeast cell membrane



Alcoholic Fermentation

Uptake of glucose:
Glucose ---> glucose-6P

Glucose-6phospate to fructose-1,6 diphosphate

FIRST STAGE - 2 ATP

Alcoholic Fermentation

frucose-1,6-diphosphate ---> dihydroxyacetone-P+ glyceraldehyde-3-P
aldolase

dihydroxyaceton-P---> glyceraldehyde-3-P
triosephosphate isomerase

glyceraldehyde-3-P + NAD + Pi ---> 1,3-diphosphoglyceric acid + NADH+H^+
triosephosphate dehydrogenase

NET GAIN: + 2 NADH+H^+

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Alcoholic Fermentation

1,3-diphosphoglyceric acid + ADP ---> 3-phosphoglyceric acid + ATP
3-phosphoglycerate kinase

3-phosphoglyceric acid ---> 2-phosphoglyceric acid
phosphoglyceromutase

2-phosphoglyceric acid ---> PEP + H2O
enolase

PEP + ADP ---> pyruvic acid + ATP
pyruvate kinase

CH3-CO-COOH CH3-COH CH3-CH2OH
pyruvic acid ---> acetaldehyde + CO2 + NADH2 ---> ethanol + NAD
pyruvate decarboxylase *alcohol dehydrogenase*

	- 2 NADH+H^+
SECOND STAGE	+ 4 ATP
NET	2 ATP

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Alcoholic Fermentation - energy balance

FIRST STAGE	- 2 ATP
	+ 2 NADH+H^+
	- 2 NADH+H^+
SECOND STAGE	+ 4 ATP
NET	2 ATP

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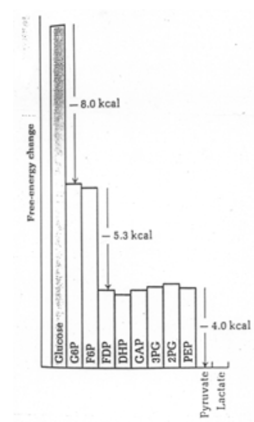
Energy barriers



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Alcoholic Fermentation

C6H12O6 --> 2 C2H5OH +2 CO2 + 2 ATP + heat
hexose ethanol gas energy

Total free energy change: -227 kcal
Conserved energy: 15 kcal (6.6%)

(aerobic) Respiration

C6H12O6 + 6O2 --> 6H2O + 6CO2 + 36 ATP + heat
hexose oxygen water gas energy

Total free energy change: -686 kcal
Conserved energy: 270 kcal (40%)

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Alcoholic Fermentation - History

C6H12O6 + 2 Pi --> 2 C2H5OH +2 CO2 + 2 ATP +heat

yeast - cause of fermentation
1632-1723 Antonie van Leeuwenhoek
1789 Lavoisier: fermentation end products ethanol and CO2
1810 Gay-Lussac: C6H12O6 --> 2 C2H5OH + 2 CO2
1818 Erxleben, De La Tour, Schwann, Kuetzing (1837): yeasts are the cause
1866 Louis Pasteur: études sur le vin, 1876 études sur la bière
Christian Emil Hansen, Hermann Müller-Thurgau, Julius Wortmann (1894)
pure culture yeast

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Biochemistry of Alcoholic Fermentation

1897 Buchner: extract of yeast retains ability to ferment glucose to ethanol
1905 Harden and Young: heat-labile fraction (zymase, proteins) + heat-stable fraction (cozymase, NAD, ATP, ADP)
Gustav Embden: cleavage of fructose 1,6 -di-P
Otto Mayerhof: verified Embden's theory and added energetics
1940: Embden-Mayerhof pathway: glucose --> pyruvate
also: Otto Warburg, CF Cori and GT Cori, J Parnas

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Alcoholic Fermentation - Uptake of Sugars

Passage through cell wall and cell membrane

3 basic entry mechanisms for uptake of solutes (nutrients) across cell membrane:
simple diffusion, facilitated (carrier mediated) diffusion, active transport

in *Saccharomyces crevisiae*, **glucose, fructose**, and mannose are transported via **facilitated diffusion**, a non-concentrative process, several carriers

sucrose is cleaved outside the cell ---> glucose + fructose
fructofuranidase (=saccharase,=invertase)
yeast has an extracellular and intracellular invertase located on the outside of the yeast cell wall

glu and fru transported into the cell

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Alcoholic Fermentation - Regulation of Pathway

MULTISTEP REGULATION OF SUGAR FERMENTATION PATHWAY
- uptake and phosphorylation (hexokinase)
- multienzyme complex of glycolytic enzymes

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Alcohol Yield

$C_6H_{12}O_6$ -------------->	2 C_2H_5OH +	2 CO_2
1 Mol	2 Mol	2 Mol
180.16 g	2x46.05 g	2x44 g
	51.1%	48.9%

actual observed yield: 47%
220 g/L sugar x 0.47 -----> 103.4 g/L = 10.3% ethanol

why only 47%?
loss of ethanol use of C for building cells production of other products (flavors!)

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Alcoholic Fermentation Byproducts - CO2

The volume of CO2 produced is about 40 to 50 times larger than that of the fermenting must.
1 Mol of CO2 is 22.4 L (180 g/L sugar (1 Mol) ---> 44.8 L)

CO2 is poisonous!!
3-4% breathing difficulties
10% severe shortage of breath, a burning candle extinguishes at 10% CO2
15% fainting, death

Fermentation rooms must be well ventilated!

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Alcoholic Fermentation Byproducts - Heat

$C_6H_{12}O_6$ ---> 2 C_2H_5OH + 2 CO_2 + 2 H_2O + 2 ATP
1 Mol glucose (180 g) ---> 24 kcal
must with 180 g/L sugar ---> 24 kcal
220 g/L sugar --->
$\frac{220 \times 24}{180}$ = 29 kcal/L

Possible temp. increase: initial must temp, 16°C + 29 = 45°C!

Approx. 20% of the heat prod. are carried away by the escaping CO2

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Alcoholic Fermentation Byproducts - Yeast Biomass

0.8 - 1.3% of C of sugar is incorporated into yeast biomass during wine fermentation

yeast lees (incl. 2/3 grape particles): 1.5 - 3% of ferm. volume

Metabolic Byproducts of Alcoholic Fermentation

PRIMARY BYPRODUCTS
pyruvic acid, acetaldehyde, ketoglutaric acid (binding partners for SO_2!)

glycerol (4-14g/L)
dihycroxyacetone-P + $NADH_2$ ---> a-glycero-P + NAD ---> glycerol + Pi

lactic acid (0.1-0.2 g/L) (lack of thiamin to decarboxylate pyruvate to acetaldehyde)
$CH_3COCOOH$ ---> $CH_3CHOHCOOH$
pyruvic acid + $NADH_2$ --> lactic acid + NAD

acetic acid (0.2-0.5 g/L):
$CH_3COH + H_2O$ + NAD ---> $CH_3COOH + NADH_2$
oxidation of acetaldehyde to acetic acid

citric acid

Metabolic Byproducts of Alcoholic Fermentation

SECONDARY BYPRODUCTS
2,3-butanediol acetoin diacetyl
higher alcohols
these fermentation byproducts are part the cell building metabolism
(byproducts of AA synthesis valine, leucine, isoleucine)
Sulfur compounds (SO_2, H_2S, mercaptans,…)

NUTRIENT LIMITATIONS
Nitrogen (amino acids, short peptides, ammonia)
Pi (H_3PO_4)
nicotinic acid (B-vitamin) important for NAD (nicotinamid adenine dinucleotide)
thiamin (B1) (pyruvate ---> acetaldehyde), lack: accumulation of pyruvate and ketoglutarate