



A look at the history and science of the alcoholic beverage JOHN KRISINGER, DEPT. BIOLOGY & NURSING NWCC TERRACE JKRISINGER@XPLORNET.CA

The alcoholic beverage History

- Early experience with fermenting fruits likely accidental
 - Observed in animals as well
- First alcoholic beverage probably wine
- Chemical analysis of jars from a Neolithic village China
- Traces of organic compounds absorbed and preserved characteristic of fermented fruits (no actual ethanol – too volatile!)

Published in PNAS

- Analysis: residue fermented drink made of grapes, hawthorn berries, honey, and rice produced in 7000–6650 BC
- Approximately same time when barley beer and grape wine were beginning to be made in the Middle East

The alcoholic beverage History

- Earliest firm evidence of wine production: 5,400 BC in Iran
- Medicinal use of alcohol mentioned in Sumerian and Egyptian texts about 2,100 BC
- Hebrew Bible recommends giving alcoholic drinks to those who are dying or depressed, so that they can forget their misery (Proverbs 31:6-7).
- "Give strong drink to him who is perishing, and wine to those in bitter distress, let them drink and forget their poverty and remember their misery no more"

Psalm 104:15 (Old Testament)

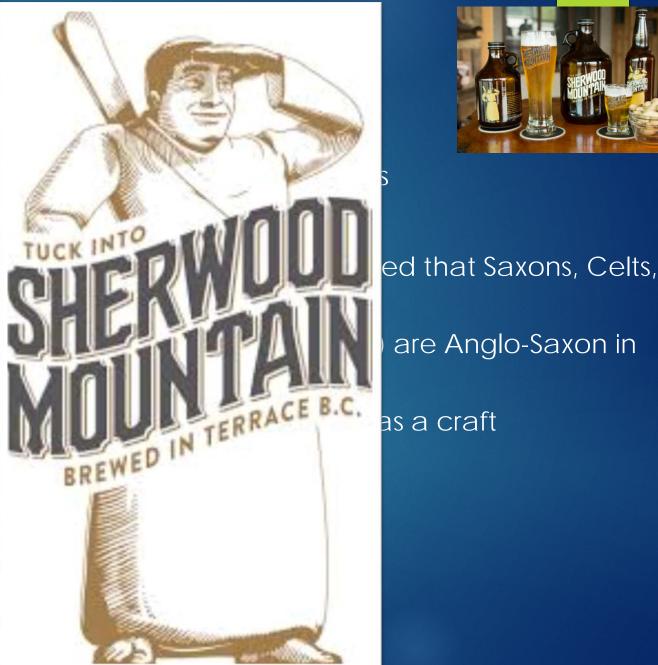
So that he may bring forth food from the earth, And wine which makes man's heart glad,....

Acts 2:15 (New Testament):

- These people are not drunk, as you suppose. It's only nine in the morning!
- Evidence for the 5' o clock rule?

The Beer History

- Before 6,000 BC beer made in Sum
- Reliefs on Egyptian tombs dating fr
- Techniques came to Europe from t
- Roman historians Pliny (23-79 AD) a and Nordic and Germanic tribes d
- Many of the English terms used in b origin
- During the Middle Ages the monas





are Anglo-Saxon in

as <u>a craft</u>

The Beer History

- Europe during <u>Middle Ages</u> beer, often of very low strength everyday drink for all classes and ages of people
- A document from that time mentions <u>nuns</u> having an allowance of six pints of ale each day (2.8 I = 8 beers!)
- Cider(apples) and pomace(mainly grape skin after juice removal) wine were also widely available
- Grape wine was the prerogative of the higher classes



Benedictine *Weihenstephan* Abbey, founded 725, oldest still operating brewery in the world (1040) 1,000th anniversary coming up







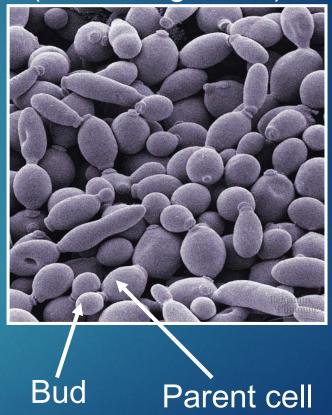


Yeasts: Unicellular Fungi

 Yeasts reproduce by <u>fission</u> or <u>budding</u>, allowing rapid growth

• Saccharomyces ("sugar fungus")

Saccharomyces cerevisiae (A Budding Yeast)

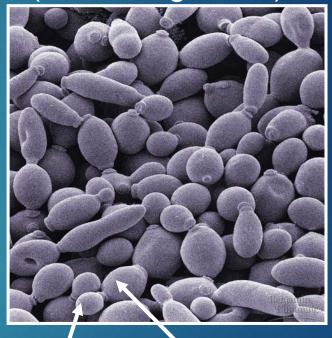


Yeasts: the Unicellular Fungi

- Yeasts reproduce by <u>fission</u> or <u>budding</u>, allowing rapid growth
- Yeasts grow in moist environments
- Saccharomyces is important in research and food production



Saccharomyces cerevisiae (A Budding Yeast)



Bud

Parent cell

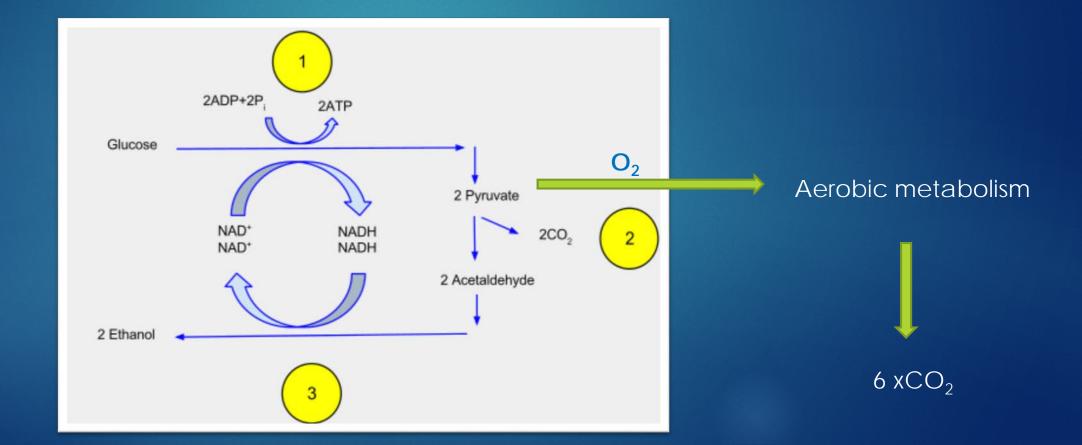
Production of Alcoholic Beverages

Many plants - high carbohydrate content
 Fermentation with yeast (low O₂ levels – yeast switches to ethanol fermentation)
 Possible up to10-18% ethanol



Alcoholic fermentation in yeast

▶ If sufficient O_2 – glucose completely oxidized to CO_2



Alcoholic fermentation in yeast

Insufficient O₂ – Fermentation

- "Emergency metabolism" to re-generate NAD⁺ ethanol is a by-product
- FYI: glucose: 4 Cal/g ethanol: 7Cal/g "wasted" in the eyes of the poor yeast (pun intended) 2ADP+2P 2ATP No O₂ Glucose Aerobic metabolism 2 Pyruvate NAD' NADH 2CO, NAD⁺ NADH 2 Acetaldehyde 2 Ethanol 6 x CO₂ 3

History of grape vine

- Wild grapes native to Armenia, Azerbaijan, Georgia, Levant, Turkey, Iran
- With invention of pottery (11,000 BC) and sedentary life style, agriculture and wine domestication
- Wine production possible
- First firm record: Georgia 6,000 BC

History of grape vine

- Widespread use in ancient world
- As beverage, medicine and ceremonial drink
- Middle Ages, wine the common drink of all social classes in the South, where grapes were cultivated (30-50° latitude)
- In the North few if any grapes were grown, beer and ale usual beverages
- Wine necessary, the celebration of the Catholic Mass

History of grape vine

- Benedictine monks one of the largest producers of wine in France and Germany
- Vineyards in Champagne, Burgundy, and Bordeaux (France 1792)
- Rheingau and Franconia (Germany 1815)
- 1435 Count John IV wealthy member of the Holy Roman Empire first to plant Riesling, the most important German grape

Great French Wine Blight



- Severe blight in 19th century destroyed many vineyards in France & nearby European countries
- Caused by an aphid (Daktulosphaira vitifoliae), originated in North America
- Transferred by ship in1850s

Grafting of resistant American stock to French scion – still done today in nearly ALL vineyards worldwide (exceptions in Chile & South Australia)

Grafting

Common technique in horticulture

- Tissues from one plant inserted into another joining vascular tissues
- One plant selected for its roots (stock)
- Other plant selected for its stems, leaves, flowers, or fruits (scion)
- Common in vineyards
 - French grapes, American roots
- Grafting used for thousands of years





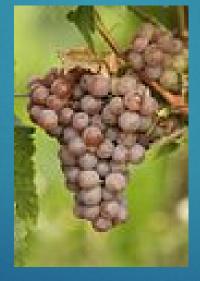
Wines

Typically grown on poor soil (compared to other crops) most species: Genus Vitis sp. Climate, weather, elevation, aspect

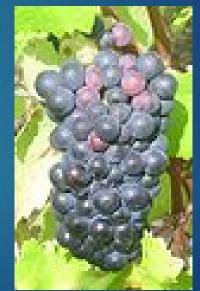
- ► White
 - May be made from non-colored or black-skinned grapes (skin removed upon pressing) fermentated at 12-15°C



Riesling (Rhein Germany)



France



Gewuerztraminer

Pino Noire France

Wines

Red

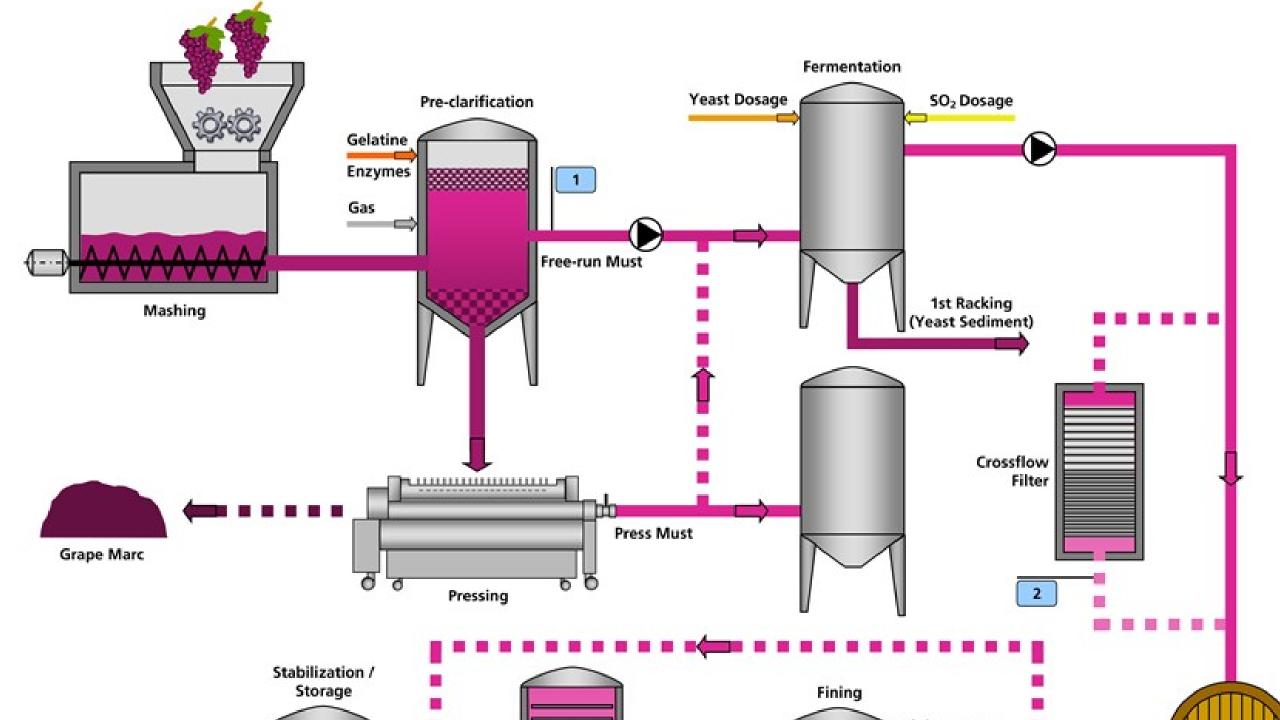
- 100s of varieties worldwide
- Black-skinned grapes skin and seeds left in contact throughout fermentation (20-29°C)
- Extraction of anthocyanins & phenolics (tannins) form skin & seeds

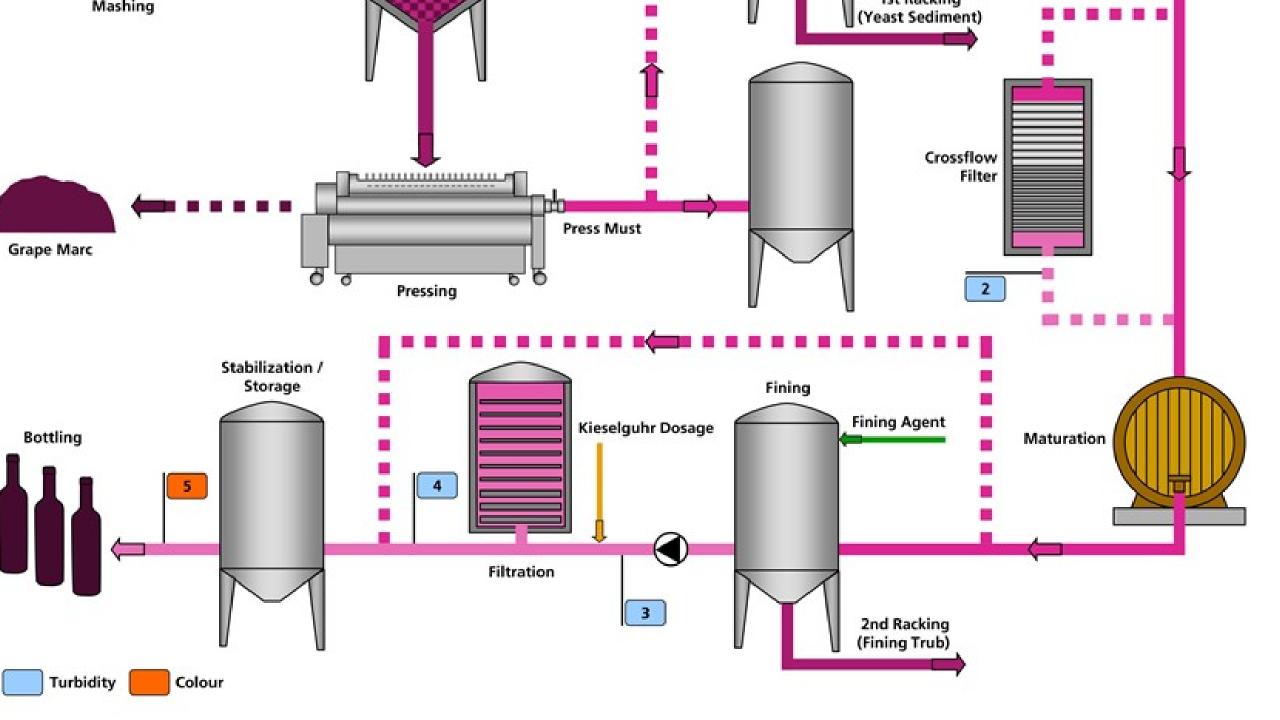
Rosé

- Produced with skin contact method
- Black-skinned grapes crushed and skins allowed in contact with juice 1-3 days duration determines final color
- Must pressed & skins discarded followed by fermentation

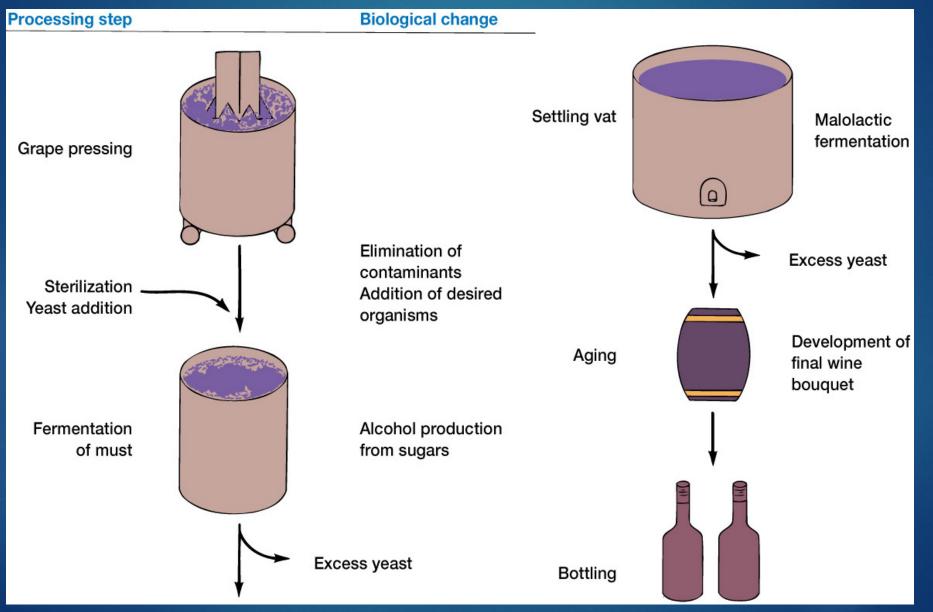
Modern production of wine

Wine starts from GRAPE JUICE! (simple beginning)
 Complex fine tuning and during and after fermentation
 Time of harvest crucial, ripening stops after harvest
 Beer starts from MALT (complex starting material)
 Less sophisticated fine tuning during and after fermentation



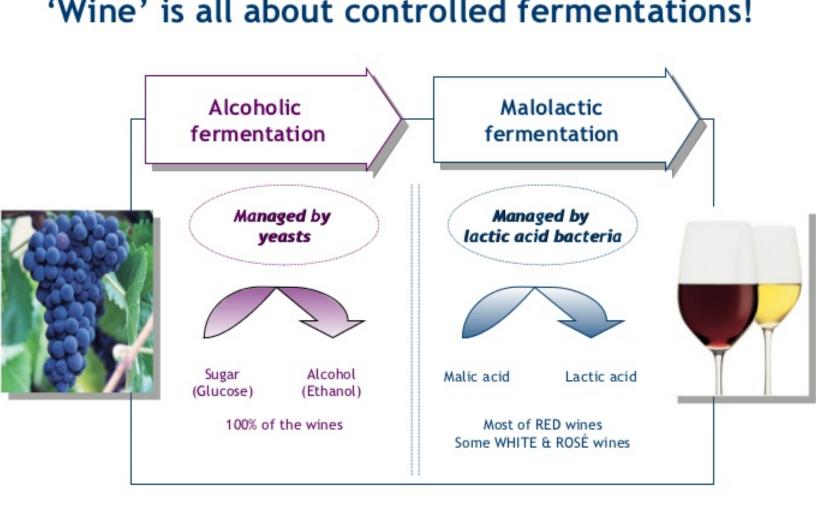


Wine Production



Why a 'Malolactic Fermentation' (MLF)?				
	MALIC ACID LACTIC ACID FERMENTATION FLAVOURS			
Microbial stability	Malic acid is a substrate for different species of wild bacteria: Oenococcus, Pediococcus, Lactobacillus Its consumption stabilizes the wine avoiding fermentation to occur in bottles and/or spoilages and wine downgrading			
Flavours & mouth-feel	Malic Acid gives a harsh sensation in wines. Its conversion by fermentation into Lactic Acid decreases acidic sensation in mouth: pH increases, mouth-feel is softer and rounder while producing additional typical and classical fermentation flavours: fresh cream, butter, crème caramel			





'Wine' is all about controlled fermentations!

CHR_HANSEN

Use of Sulfur Dioxide (SO₂)

Used by the Romans in winemaking, discovered that burning sulfur candles inside empty wine vessels keeps them fresh and free from vinegar smell (acetic acid)

Antimicrobial and anti-oxidant properties

Prevents growth of acetic acid bacteria

Wine aging

Very complex chemistry changing composition of wines

Influence of vessel (barrels)

Exposure to oak during fermentation or after (during barrel aging) more phenolic compounds

Aging continues in bottles

Aging in Oak barrels



Toasted oak barrels

	LIGHT TOAST	MEDIUM TOAST	HEAVY TOAST
AMERICAN OAK	vanilla, dill, coconut	vanilla, honey, cara- mel, toast, roasted nut aromas, strong coconut, roast cof- fee, and cocoa	strong roast coffee, espresso, caramel- ized sugar, tiramisu, wood smoke and vanilla
FRENCH OAK	vanilla bean, cara- mel, holiday spice flavors like nutmeg, clove, allspice and dried ginger	cedar, cigar box, milk chocolate and baking spice	crème Brûlée, cedar, charcoal, and Asian spices like cinna- mon, ginger and clove
HUNGARIAN OAK	vanilla, herbal fla- vors, sweet spice flavors like clove and cinnamon	stronger butter- scotch, banana, sar- saparilla and sweet spice	strong spice, vanilla, butterscotch, toffee and molasses

Wine's sweetness

At harvest b/w 15 -25% sugar content of grapes simple sugars (glucose & fructose)

- Very high sugar content kills yeast via (high) alcohol content during fermentation
- No wine ever fermented completely "dry" (meaning without any residual sugar)

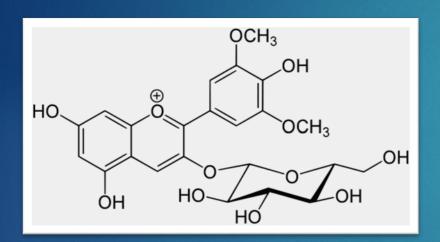
Wine's sweetness

At harvest [glucose] = [fructose] Grape over ripened [glucose] < [fructose] sweeter!

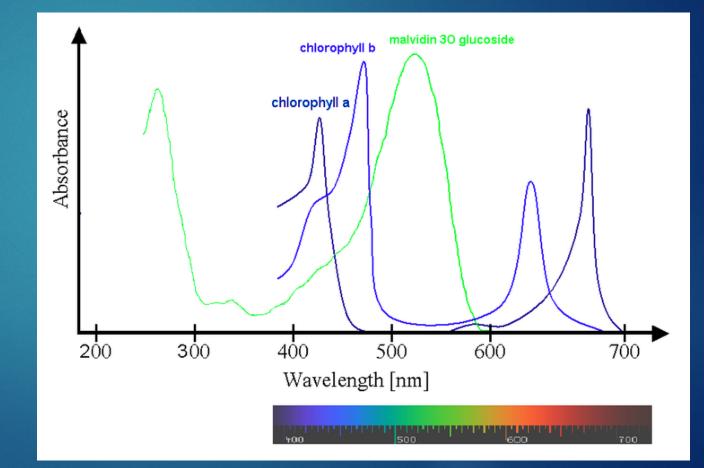
Dessert wines

- glucose fermented first
- Fermentation can be stopped (either by temperature control or the addition of ethanol (fortification) wine high in fructose

Major anthocyanin in black grapes: Malvidin-3-glucoside (Oenin)



Members of the class of Flavonoids (plant secondary metabolites common in fruits & vegetables)



Does drinking red wine protect against heart disease ?



Does drinking red wine protect against heart disease ?



Modern day production of beer

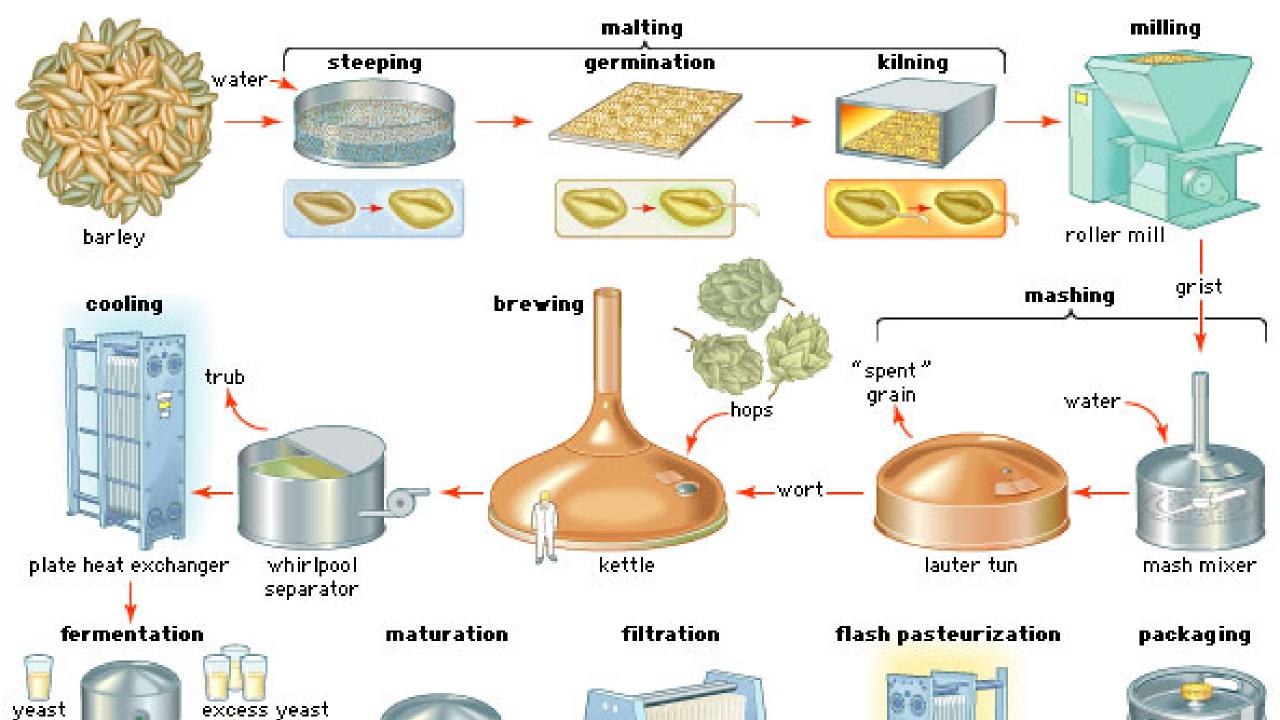




American Bud: made with 30% rice

Czeck Republic (town of Budvar)

German (Bavarian) Purity Law: 1516: Beer shall contain ONLY: Water, barely and hops 500th anniversary here!



Malting

Barley seeds allowed to germinate as in nature

Steeping

- immersing barley in water at 12 to 15 °C 40 to 50 hours
- grain imbibes water white root sheath breaks through the husk

Germination

Root embryo - gibberellic acid - synthesis of a-amylase – conversion of starch into sugars

Proteases & β-glucanases

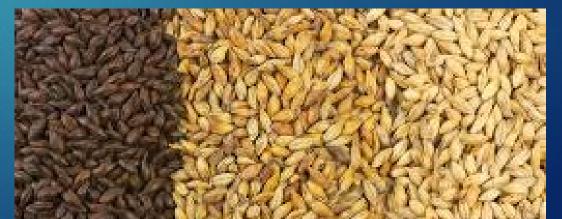
proteins & complex sugars into soluble amino acids and glucose



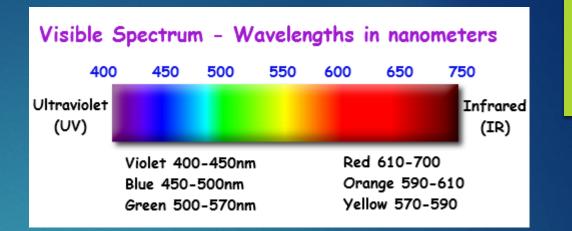
Malting

Kilning

- Green malt dried 5 % moisture in lager & 2 % ale malts
- Drying arrests enzyme activity preserving 40 -60 % in an active state
- ► Curing at higher temperatures → reaction between amino acids and sugars → melanoidins (Maillard Reaction)- colour and flavour to malt
- Kilning high flow of dry air at 50 °C for lager malt and 65 °C for ale malt
- Temperature rising to 70–75 °C
- Curing stage: temperature to 75–90 °C for lager and 90–105 °C for ale
- Finished malt screened to remove rootlets



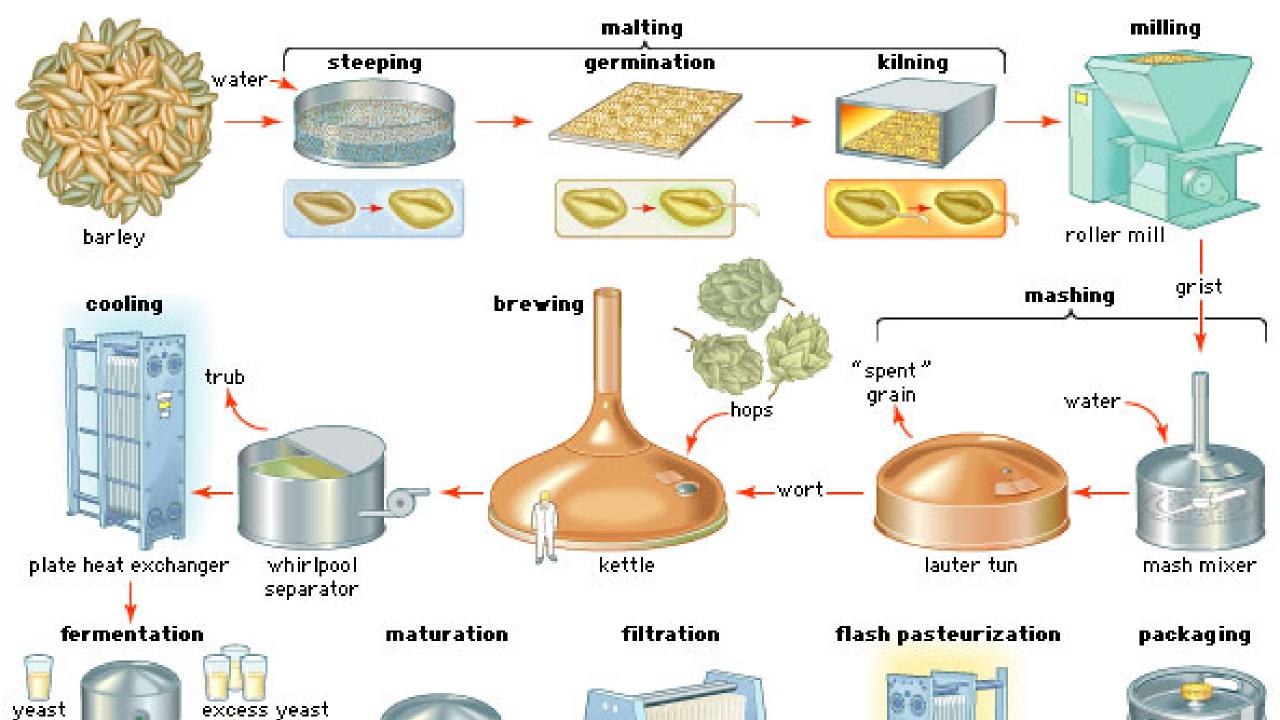
Beer colour



Standard Reference Method

Light absorption @ 430 nm (blue/purple) * 12.7

SRM	STYLE	COLOR
2	PALE LAGER	
3	MAIBOCK	
4	WEISSBIER	
6	PALE ALE	
8	Saison	
10	ESB	
13	DOUBLE IPA	
17	Dark Lager, Amber Ale	
20	BROWN ALE, DUNKELS	
24	DOPPLEBOCK, PORTER	
29	Stout	
35	BALTIC PORTER	
40+	Imperial Stout	



Mashing



- In the brewery malted grain milled to fine particles
- Mixed with hot water right mix of salts
- Fine ales need high levels of calcium
- Famous pilsners are low levels of calcium
- 3:1 water : malt 65°C
- Granules of starch more susceptible to enzymatic digestion

Mashing



- Amylase digestion of starch (about 1 hr)
- Some brewers add starch from other sources: corn or rice (adjuncts) (☺)
- Liquid portion of the mash becomes: wort
- Recovered, either by straining through residual spent grains or by filtering through plates
- Wort: about 10 % sugar (mainly maltose and maltotriose), amino acids, salts, vitamins, carbohydrates, and small amounts of protein

Lautering



- "Lautering" Old German word for Purifying
- Mashout Temperature to 77 °C
- Stops amylase activity
- Makes mash and wort more fluid
- Recirculation of wort from bottom adding to the top
- Lauter tuns typically with slotted bottoms to assist filtration process
- Mash functions as a filter to capture mash debris and proteins
- Step monitored via turbidimeter

Brewing



- Wort run to kettle ("copper")
- Boiled for I hour
 - Sterilisation of wort
 - Precipitation of proteins (can cause cloudiness)
 - Removing unpleasant grainy characters of barley
 - Some adjunct sugars & some hops can be added here

Hops

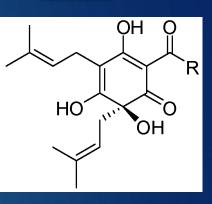


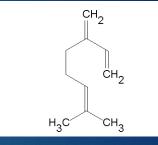
- Varieties hop (Humulus lupulus) selected and bred for the bitter and aromatic qualities
- Female flowers, or cones glands containing flavoring compounds
- Traditionally dried hop cones added
- Powdered compressed hops often used more efficiently extracted
- Hop extracted made with liquid CO₂ extraction
- Member of the Cannabaceae family (③)

Hopping

- Two components:
- <u>Resins (a-acids)</u> 'isomerised' during boiling iso-a-acids
 - bitterness
 - process inefficient, today extracted hop oils may be used
 - Hop is expensive!
- Oils 'hoppy nose' of beer
 - Very volatile (hops added before boil loss of aroma)
 - Added towards the end of boiling
- Traditional ale production some hops added at end of the process (complex mixture of oils distinctive character)
- Dry hopping

Myrcene





Humulene





Plant world full of terpenes

- Geraniol (Geranium)
- Limonene (Lemons)
- Menthol (peppermint)
- Thymol (Thyme)
- Taxol (Pacific Yew)
- Retinol (carots)
- Lycopene (tomatoes)

- Natural rubbers
- Camphor (camphor laurel)
- Borneol (Artemisia absinthium, etc.)
- Eucalyptol (Eucalyptus tree)
- Zingiberene (Ginger)
- Caryophyllene (Cloves)
- Cholesterol (animals) C-30

International Bitterness Units

- American Lager: 5
- Blonde ale: 15-30
- Kölsch: 18–25
- Märzen/Oktoberfest: 18–25
- Ordinary English bitter: 20–35
- Porter: 20–40
- Brown ale: 15–25, North American styles 25–45
- Bohemian-style Pilsner: 30–45, range up to 100 (e.g., German Bitterpils)
- India Pale Ale: 40 or higher
- An Irish stout like Guinness: 25–60





Fermentation



- Hopped wort cooled and pitched with yeast
- Many strains of brewing yeast (Saccharomyces cerevisiae)

Yeast strains

- Saccharomyces
- Ale strains surface fermenting S. cerevisiae
 - few days at temperatures up to 20°C
- Lager strains bottom fermenting S. carlsbergensis
 - lager fermentations as low as 6°C can take several weeks
- Both types need some oxygen
- Traditional ale brewing beer mixed with hops, some priming sugars and with isinglass finings (from the swim bladders of fish to settle out the solids in the cask (mainly collagen gelatine)
- Traditional lager brewing the 'green beer' matured by several weeks of cold storage, prior to filtering
- Modern yeast systematics, brewing strains "S. cerevisiae"



Fermentation



- Pitching temperature of wort: <u>15 to 18 °C for ale & 7 to 12 °C for lager</u>
- During fermentation specific gravity \downarrow sugars \rightarrow alcohol
- Yeast multiplies five- to eightfold and generates heat
- Temperature allowed to reaches 20 to 23 °C for ale & 12 to 17 °C for lager
- Cooling follows to 15 °C for ale & 4 °C (39 °F) for lager
- Near end of fermentation green beer most yeast removed
- Still containing about 500,000 yeast cells/ml
- Secondary fermentation

Secondary fermentation



- Slow secondary fermentation of residual or added sugar
- Generates CO₂- vented <u>purging green beer of undesirable volatiles</u>
- Yeast activity removes strong flavouring compounds such as <u>diacetyl</u>
 - At moderate conc. Buttery flavor high level butterscotch flavor undesired in beer

Sealed vessels then increases carbonation giving the beer its "condition"

Conditioning - Carbonation



- Relatively short conditioning period after fermentation and before filtration
- At -1°C for a minimum of three days
- More proteins precipitate beer less likely to turn cloudy
- Filtered beer adjusted to the required carbonation
- FYI J. J. Schweppe (1740–1821) developed manufacture of carbonated mineral water Schweppes Company in Geneva 1783
- \blacktriangleright 1767, Joseph Priestley discovered a method of infusing water with CO₂

Preservation



- Beer kept oxygen free (which ultimately spoils beer)
- Filtered through cellulose or diatomaceous earth to remove all yeast
- Packaged at 0 °C under pressure of carbon dioxide
- Most beers packaged in bottles or metal cans pasteurized 60 °C for 5 20 min





The final product at last!



- Several hundred of simple organic compounds characterized in beer
- Majority of these are produced by yeast
- Bitter substances of hops, ethanol, and CO₂ have the greatest effects
- Other compounds: esters: isoamyl acetate (banana), ethyl hexanoate (apple), and ethyl acetate (solvent)
- Higher alcohols: isoamyl alcohol and 2-phenyl ethanol
- Acids: octanoic, acetic, isovaleric, butyric, malic, and citric
- Dialkyl sulfides: dimethyl sulfide
- Diketones: diacetyl
- Ethyl isovalerate (ester) & nonenal (aldehyde) contribute to stale and oxidized flavours

A six pack of nutrition (21 volume)

- 540 mg K RDI: 3,500 mg
- 80 mg Ca RDI: 1,000 mg
- 280 Mg P RDI: 1,000 mg
- 10 g protein* RDI: 50g
- 70 g carbohydrate RDI: 300 g
- 820 Cal "RDI" 2,000 Cal





A six pack of nutrition (2 I volume)

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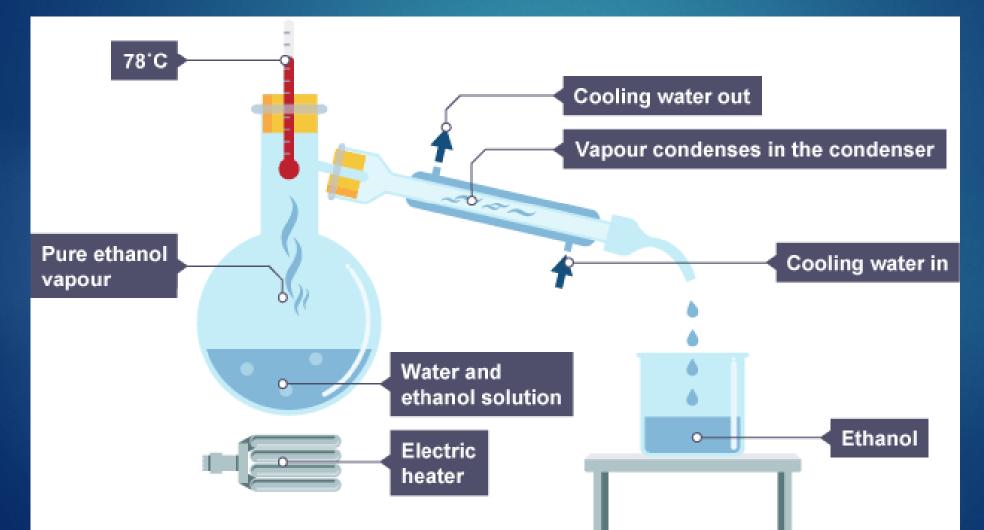




Distillation of alcohol

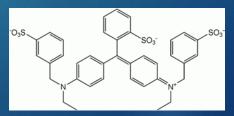
- First evidence Greek alchemists working in Alexandria the 1st century AD
- First dated and certain evidence of distillation of alcohol School of Salerno in the 12th century
- Fractional distillation developed by Taddeo Alderotti in the 13th century
- 1500 German alchemist Hieronymus Braunschweig published: Liber de arte destillandi (The Book of the Art of Distillation)

Principle of distillation Bp: H₂O 100° C ethanol 78° C



Distilled Spirits

- Fractional distillation used to increase ethanol concentration to any level up to 95% ("190 proof")
- Most are kept at 40%
- Storing aging
- All distillates of alcoholic fermentations are clear/colorless
- Storing in wooden barrels
 - Leaching of compounds from wood into products
 - Color, added flavour



Classic distillation equipment



Apple Jack – Freeze distillation

Fermented apple juice (10% ethanol)

- Simply left in barrels outside to freeze
- Periodically removal of ice forming on surface
- Up to 30-40% ethanol
- Illegal as is hot distillation (moonshining)
 - Potential of methanol poisoning
 - Distillation may lead to concentration of toxic methanol
 - In mere homemade beer & wine low level methanol contamination possible but not enriched as in distillation

Home distillation

- Moonshine, white lightning, mountain dew, hooch, homebrew, and white whiskey terms used to describe highproof distilled spirits that are generally produced illicitly
- During the Prohibition many intoxications via methanol and other chemicals from home made stills (lead, glycol from radiators used as condensers etc.)





Distilled beverages

Names like "life water" have continued to be the inspiration for the names of several types of beverages

- Gaelic whisky, French eaux-de-vie and possibly vodka
 - 1715, from Gaelic uisge beatha "whisky," literally "water of life"
- Scandinavian akvavit spirit
 - named from the Latin phrase aqua vitae



 Grain spirit, whisky specific origins are unknown
 Production in Ireland and Scotland for centuries
 First confirmed written record of whisky: 1405 in Ireland

From malted barley mentioned in Scotland in 1494

Gin

- Distilled spirit flavored with juniper berries added during distillation
- known as Jenever (the Dutch for "juniper)"
- Originally used for medicinal purposes



Brandy



- Originated in 15th century in wine growing regions of Eurasia
- Distillate aged in oak barrels to mature

Vodka



Most from sorghum, corn, rye or wheat

from potatoes, molasses, soybeans, grapes, rice, sugar bees etc.

Tequila

- From blue agave plant area surrounding the city of Tequila
- After harvesting, slowly baked in ovens to break down complex fructans into simple fructose
- shredded or mashed



- Some pulp fiber, added to fermentation tanks for a stronger agave flavor
- Agave juice fermented, distilled twice
- Clear "silver" tequila
- Aged in wooden barrels developing a mellower flavor and amber color





Problem of alcohol abus

Acute intoxication problem

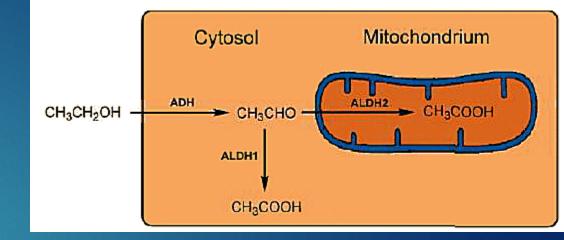
- Euphoria, risk taking bahvi etc.
- Sedation, unconsci
- Chronic problem
 - Social, finar

Fatty live

- r cirrhosis, acute pancreatitis
- Alcological control of the desire of the desi

- on, emesis, falls, trauma,
- ratory arrest) (death)

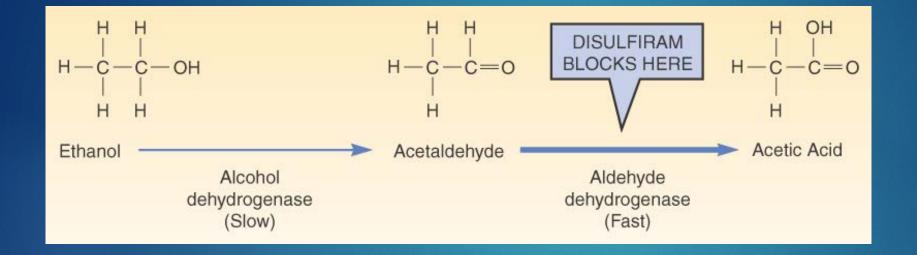
Pharmacokinetics of alcohol



Metabolism

- Hepatic <u>alcohol dehydrogenase (ADH)</u> oxidation to acetaldehyde oxidized by <u>acetaldehyde</u> <u>dehydrogenase (ALDH)</u> into acetic acid - Acetyl-CoA – Krebs cycle or lipogenesis (7kcal/g!) "beer belly"
 - ► ALDH2 gene mutation $G \rightarrow A$ (Glu \rightarrow Lys substitution) in many Asian people (50% of Japanese origin!)
 - 500-fold decrease in effectiveness of acetaldehyde dehydrogenase activity – increased toxicity due to acetaldehyde accumulation

Antabuse® Disulfiram

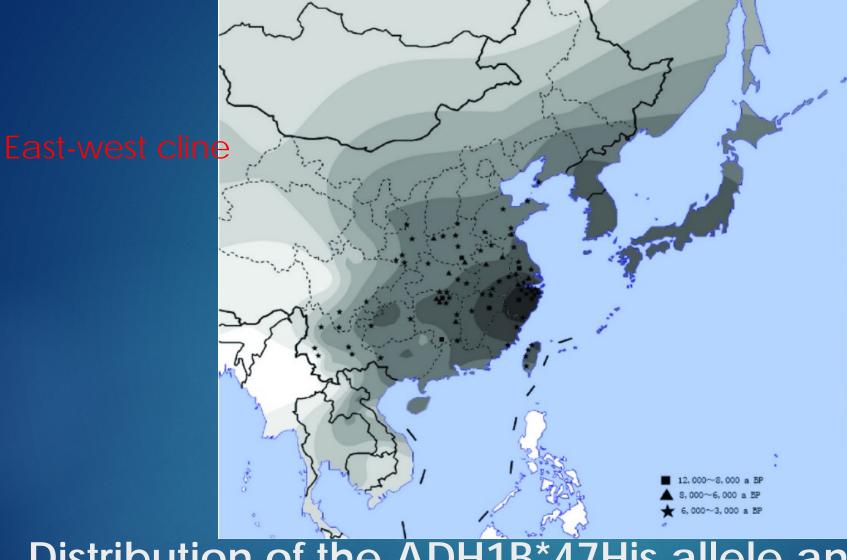


Disulfiram aversion therapy "Abhorrierende Alkohol Entzugs Therapie" (©)

ADH 1B Polymorphism

Alcohol dehydrogenase single aa mutation: Arg → His
Enzyme <u>40-100 fold increased</u> oxidation of ethanol
Accumulation of acetaldehyde – avoidance of alcohol
Variant appeared in Southern China about 7-10 k years ago
Positive selection since cultivation of rice and fermented alcoholic foods and drinks

Strong east - west cline of frequency



Distribution of the ADH1B*47His allele and the sites of early rice relics

North America's history of alcoholic beverages

- Prior to contact with colonists, alcohol use and production very limited to Southwestern US
- weak beers, wine and other fermented beverages with low alcohol concentrations (8%-14%)
- used only for ceremonial purposes

North America's history of alcoholic beverages

- In Colonial America Europeans introduced widespread consumption of alcohol
- Mayflower brought more beer than water as it departed for the New World
- Drinking wine and beer at that time was safer than water
- Alcohol used as analgesic, provided energy necessary for hard work, and generally enhanced the quality of life
- Early traders caused a large demand for alcohol used to trade for animal skins and other materials
- Traders giving free alcohol to the Native Americans during trading sessions

Ethnic (Genetic) links to alcoholism?

- Like nearly all complex medical conditions, drug & alcohol addiction displays a "genetic predisposition"
 - Hypertension, obesity, depression, schizophrenia, etc.
- 2013 review of academic literature on the issue,
- In there is a "substantial genetic component in Native Americans" but that these factors are "similar in kind and in magnitude to the genetic influences contributing to the liability for these phenotypes in other ethnic groups." American Journal of Psychiatry 2013
- Genetic variants in dopamine receptors most likely one of the culprits