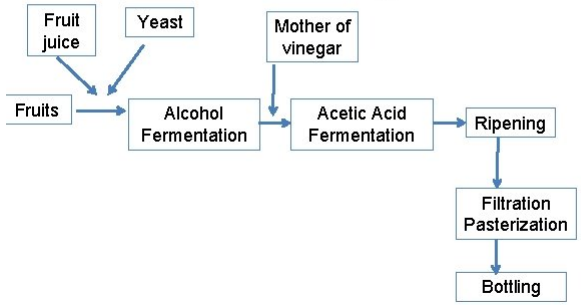
FDE 437
FERMENTATION TECHNOLOGY

Vinegar Production

Common production flowsheet of vinegar



Vinegar

- **Vinegar** is a product resulting from the conversion of alcohol to acetic acid by acetic acid bacteria, *Acetobacter* spp.

- **Vinegar** may be defined as a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation.

- **Vinegar** is a dilute solution of acetic acid produced by a two-stage fermentation process.

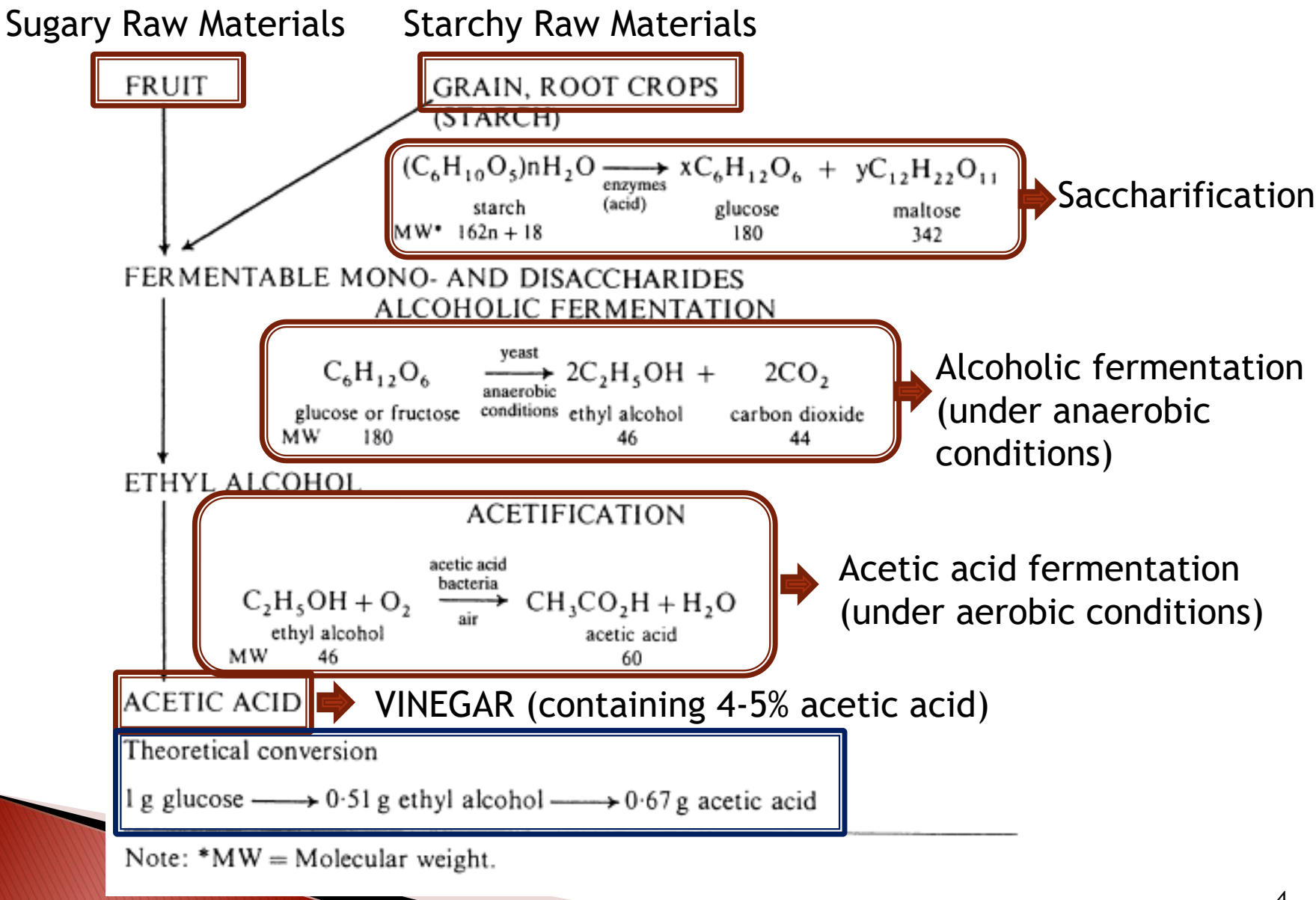
1. In the first stage, fermentable sugars are converted into ethanol by the action of yeasts, normally strains of *Saccharomyces cerevisiae*.

2. In the second stage, bacteria of the genus *Acetobacter* oxidize the ethanol to acetic acid.

Vinegar

- **Vinegar** is a product containing 4-5% acetic acid, traditionally produced by acetic acid fermentation of dilute alcoholic solutions.
- At the present time, it is produced microbiologically from natural alcoholic solutions or by dilution of acetic acid.
- **Vinegar** has been known since ancient times, for more than 10 000 years, and has been used for its typically acid flavor.
- It uses were many and various for centuries, not only for food but also for medicinal and ritual uses.
- It is traditionally the product of the acetic fermentation of dilute alcoholic liquors and it was wine that was the first alcoholic liquid used, thus the derivation of its name (from the French *vin aigre* = sour wine).
- The term «vinegar» is derived from the French words *vin* (wine) and *aigre* (sour).
- Generally, vinegar is classified as a condiment that contains a minimum of 4% (w/v) acetic acid.

Schematic outline of vinegar production



Vinegar is the product of the acetic fermentation of slightly alcoholic liquids (less than 10–12% by volume of ethyl alcohol); transformation of alcoholic liquids into vinegar is not really fermentation, but oxidation.

1. Step (Alcoholic fermentation):

- Normally the yeast used in alcoholic fermentation is a strain of the species *Saccharomyces cerevisiae*.
- The transformation of a hexose by S. *cerevisiae* can be represented chemically by the Gay-Lussac equation:

$$\underset{\text{fermentable hexose (180 g)}}{C_6H_{12}O_6} \rightarrow \underset{\text{ethanol (92 g)}}{2C_2H_5OH} + \underset{\text{carbon dioxide (88 g)}}{2CO_2}$$

2. Step (Acetic acid fermentation/Acetification):

- Acetification is the oxidation of ethanol by bacteria to produce acetic acid and water. The process can be represented chemically by:

$$\underset{\text{ethanol (46 g)}}{C_2H_5OH} + \underset{\text{oxygen (32 g)}}{O_2} \rightarrow \underset{\text{acetic acid (60 g)}}{CH_3COOH} + \underset{\text{water (18 g)}}{H_2O}$$

- From the stoichiometry of the equation it is apparent that 1 litre of ethanol should produce 1.036kg of acetic acid and 0.313 kg of water. The slight increase in volume that occurs during fermentation in the range of 1- 3% for the ethanol concentrations generally used means that approximately 1 % (v/v) of ethanol should produce 1 % *(w/v)* of acetic acid. This relationship, which holds best at ethanol concentrations around 9.2% (v/v), is used as the basis for forecasting the eventual acidity of the vinegar and calculating the fermentation yield.

Acetic Acid Fermentation Pathway

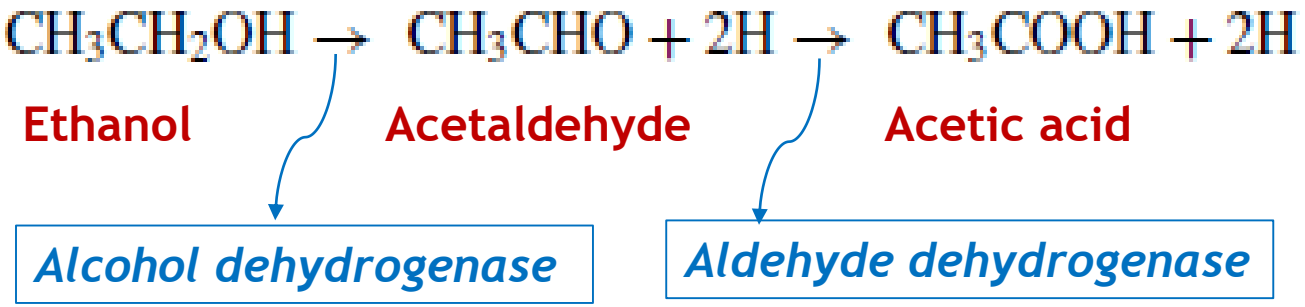
- Acetic acid fermentation is the biochemical process by which *Acetobacter* spp. oxidise ethanol into acetic acid **under strict conditions of aerobiosis**.
- Two genera of acetic acid bacteria are known in the production of vinegar: ***Acetobacter*** and ***Gluconobacter***.
- The acetic acid pathway used by *Acetobacter*, *Gluconobacter* and other acetic acid bacteria is an example of what biochemists refer to as **an incomplete oxidation**.
- In most oxidative pathways (e.g., the Krebs or citric acid cycle), organic substrates are ordinarily oxidized all the way to CO_2 and H_2O.
- However, in the vinegar fermentation, acetic acid bacteria usually oxidize the substrate, ethanol, only to acetic acid. There are exceptions that exist where complete oxidation to CO_2 can occur.

Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)

- Transformation of ethanol into acetic acid is the most well-known characteristic of acetic acid bacteria.

- This transformation involves two biochemical reactions: ethanol is first transformed into acetaldehyde in a reaction catalyzed by alcohol dehydrogenase and the acetaldehyde is then transformed into acetic acid by aldehyde dehydrogenase.

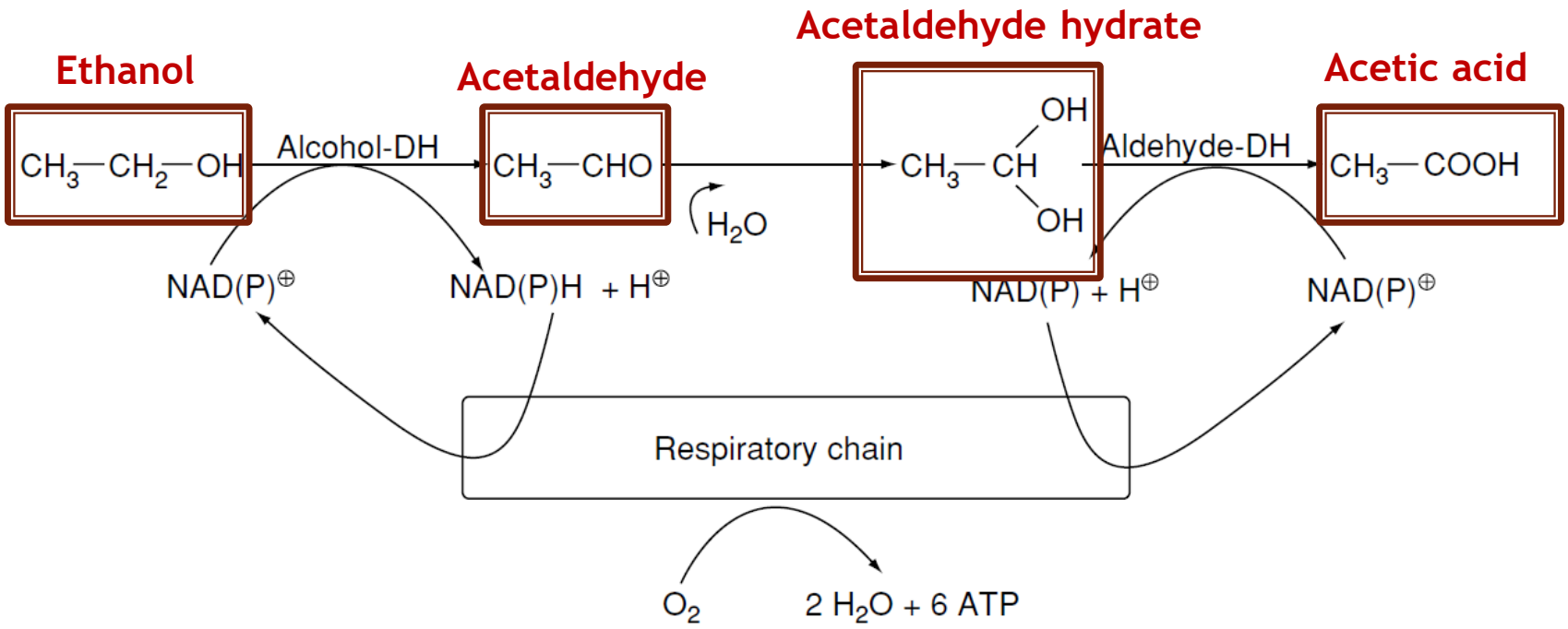
- Ethanol oxidation is performed by two sequential reactions:



Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)

- Briefly, the acetic acid pathway consists of just two main steps.
- First, ethanol is oxidized to acetaldehyde.
- Then, the acetaldehyde is oxidized to acetic acid.
- An intermediate step, in which acetaldehyde hydrate is formed, may also occur.
- Oxygen is required as the terminal electron acceptor.
- It should also be noted that the conversion of ethanol to acetic acid occurs on an equimolar basis, such that after the reactions are complete, the final concentration of acetic acid will be equal to that of the ethanol in the starting material (assuming negligible loss from evaporation).
- Only a minor amount of the carbon from ethanol is used for biomass or converted to other products.

Acetic Acid Fermentation Pathway (Oxidation of Ethanol to Acetic Acid)



Overoxidation

- In addition to their ability to oxidise ethanol, *Acetobacter* and *Gluconacetobacter* species can further oxidise acetic acid to CO_2 and H_2O, generating the so-called **acetate overoxidation**, that is carried out by the tricarboxylic acid cycle when there is a high level of dissolved oxygen and no ethanol in the medium.

- Strains of *Gluconobacter* are not able to overoxidise because of nonfunctional α-ketoglutarate dehydrogenase and succinate dehydrogenase of tricarboxylic acid cycle; they can only oxidise ethanol to acetic acid.

- Because entry into the acetate cycle is inhibited by the presence of ethanol, it is essential to maintain a low concentration of ethanol in the presence of acetic acid bacteria to prevent this full oxidation.

- **In order to prevent over-oxidation, ethanol concentrations between 0.5 and 1% are regularly maintained in vinegars**.

Acetic Acid Bacteria (AAB)

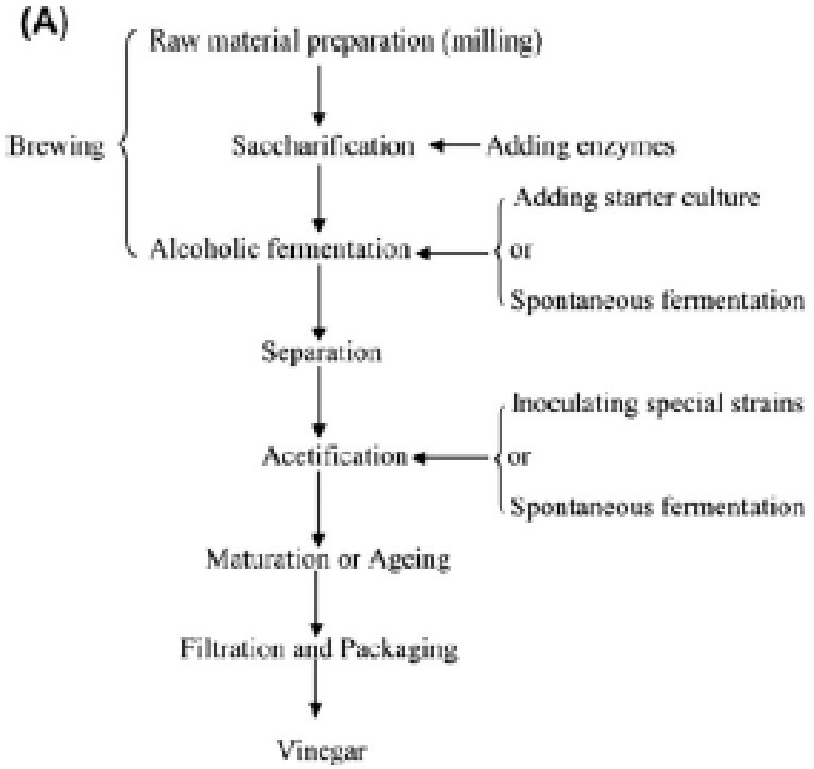
Strains of acetic acid bacteria to be used in industrial production should

a) tolerate high concentrations of acetic acid;
b) require small amounts of nutrient;
c) not overoxidize the acetic acid formed;
d) be high yielding in terms of the acetic acid produced.

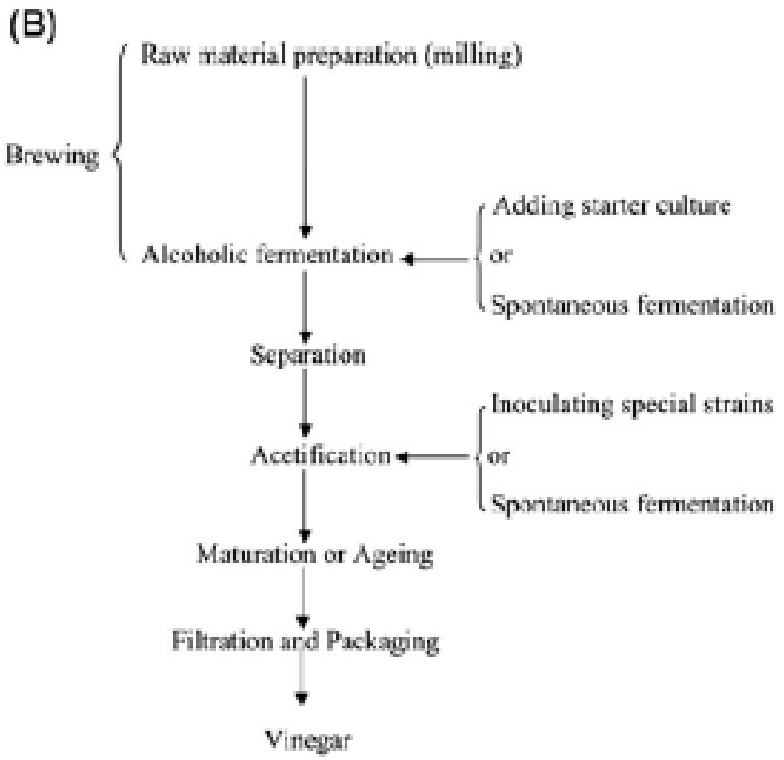
- **Over-oxidation** can occur and it is undesirable. In over-oxidation acetic acid is converted to CO_2 and H_2O. It occurs when there is a lack or low level of alcohol.

Manufacturing process of vinegar

Common procedure for vinegar production



The fermentation process of cereals vinegar



The fermentation process of fruits and other vinegar

Factors affecting the production of vinegar

- Air (Oxygen)
- Alcohol
- Acid
- Temperature
- Nutrients
- Water
- Inoculation

Spirit vinegar:

- Spirit vinegar, sometimes referred to as white, distilled, or alcohol vinegar, is prepared by acetous fermentation of an alcoholic distillate obtained from the products of alcoholic fermentation of natural sugar solutions.

- In countries where it is permitted by law, wide use is made of synthetic ethanol, diluted to 10–14% v/v.

- Spirit vinegar is colorless and is often colored with caramel; it is strongly acid but not aromatic; the distillation of the alcoholic liquids increases the concentration of ethanol, but reduces flavor.

- This vinegar is less expensive and is the most widespread in the world. When diluted to 4–5% acidity it is used for pickling.

Synthetic Vinegar:

- A number of countries allow a nonfermented vinegar to be used for alimentary purposes which may be produced using synthetic acetic acid that is diluted, aromatized, and colored.
- The synthetic vinegar thus obtained needs to be colored artificially, and for this purpose caramel is commonly used.
- It is then aromatized with the addition of sugars, chemical seasoning, and salt or with the addition of natural vinegar.
- The end product must contain at least 4% w/v of acetic acid, as in the case of fermented vinegars. However, the name 'vinegar' is not always accepted for this product; in the UK for example, it must be labeled 'nonbrewed condiment.'

Balsamic vinegar:

- Traditional balsamic vinegar of Modena, Italy is made from white and sugary Trebbiano grapes grown on the hills around Modena.
- The grapes are harvested as late as possible to take advantage of the warmth of the weather.
- The traditional vinegar is made from the cooked grape ‘must’ (juice) matured by a long and slow process of natural fermentation, followed by progressive concentration by aging in a series of casks made from different types of wood and without the addition of any other spices or flavorings.
- The color is dark brown and the fragrance is distinct.