# VINEGAR PRODUCTION

## Introduction

The word vinegar is derived from the French vin (wine) and Aigre (sour). Vinegar is a sour and sharp liquid used as a condiment and food preservative. Vinegar is a liquid fit for human consumption, produced from a suitable raw material of agriculture origin, containing starch, sugars or starch and sugars, by the process of double fertilise fermentation, first alcoholic and then acetous. The raw material used for vinegar production include rice, grapes, malt, apple, honey, potatoes. Vinegar is used as a food condiment, as a preservative agent and in some countries as a healthy drink.

Vinegar contain 5% acetic acid in water, varying amounts of fixed fruits acids, colouring matter, salts and a few other fermentation products which impart a characteristic flavour and aroma to the product.

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## ⇒ VINEGAR PRODUCTION

Vinegar is the product made from the conversion of ethyl alcohol to acetic acid by a genus of bacteria, Acetobacter. Therefore, vinegar can be produced from any alcoholic material from alcohol-water mixtures to various fruits wine (pepplar and Beaman 1967) Vinegar bacteria are also called acetic acid bacteria. (AAB), are members of the genus Acetobacter and characterised by their ability to convert ethyl alcohol ($C_2H_5OH$) into acetic acid ($CH_3COOH$) by oxidation.

Vinegar is a solution of acetic acid produced by a two-step bioprocess.

In First step - Fermentable sugars are transformed into ethanol by the action of Yeast.

In Second stage - AAB oxidize the ethanol into acetic acid in an aerobic process. AAB are well known for their ability to spoil wines because they can produce large amounts of acetic acid from ethand and other compounds present in wine.

## ⇒ Substrate Used In Vinegar

One of the critical steps in vinegar production is the preparation of the raw material. This step is required to obtain the fermentable sugar and juice solution to be acetified. The processing differs depending on the raw material used. In general, fruits require less preparation than seeds; however, seeds are more easily stored and preserved after harvest. Fruits are highly perishable, rich in water, and need to be processed very quickly. Therefore basic safe food handling practices, storage, and processing are essential to prevent the growth of pathogenic micro-organisms. These micro-organisms could alter the quality of the final product or even produce dangerous toxins such as aflatoxin. Processing has expanded the market of both fruit and vegetables advanced products. The seasonal gluts are avoided by the utilization of fruits/vegetables in processing industries for the preparation of various value added products. In advanced countries, 70-75% of perishables are processed before reaching the consumer's table, whereas in India only 1-2% of the total produce is pro

processed utilizing only 40% of the installed processing capacity.

## → Micro-organism Involved In The Vinegar Production

After raw material preparation, the alcoholic fermentation and acidification processes play a key role in vinegar production. Depending on the environmental factors (temperature, pH, water activity) or the nutients (carbon sources) and the microbial diversity present in the raw material, different biotransformations could takes place.

Microbial species involved in fermentations may range from yeast and lactic acid bacteria (LAB) to molds and AAB. The micro-organisms involved in the elaboration of vinegars are mainly yeasts and AAB. The former begins responsible for the alcoholic fermentation, and the latter needed for the acetification

- YEASTS

Yeasts are the most important micro-organisms during alcoholic fermentation because they influence fermentation speed, wine flavour and other wine qualities. Yeasts are defined as 'unicellular ascomycetes or basidiomycetes fungi, whose vegetative growth result predominantly from budding or fission. Yeasts do not form their sexual states within or upon a fruiting body.

In 2009 Rainieri and Zambonelli optimized alcoholic fermentation as a process where the sugar as a substrate was converted into ethanol in presence of a yeast belonging to the class Saccharomycetes; Phylum Ascomycota was responsible for fermentation. Further studies revealed that yeasts had high tolerance to acidity that facilitated their survival and growth in fruit juices that have pH values below the tolerance level for several other micro-organisms. The substrate used for yeast metabolism was mainly monosaccharides like glucose, fructose and mannose that were metabolised into two molecules of pyruvate in the glycolysis

also called Embden-Meyerhof-Parnas pathway (EMP pathway). The pyruvate was further reduced to ethanol ($C_2H_5OH$) and carbon dioxide by the enzymes pyruvate decarboxylate and alcohol dehydrogenase. The overall chemical reaction is represented as :-

$$C_6H_{12}O_6 \longrightarrow CH_3-CH_2-OH + 2\,CO_2 + 55\ kcal$$

Glucose Ethanol Carbon dioxide

Results indicated that theoretically, the yield of ethanol was about 65% of the initial glucose content, but the actual conversion efficiency was reduced to about 60% due to loss of glucose for production of minor compounds and growth.

Fleet (2003) explored that the saccharomyces genus was most commonly used in beverage industry due to their higher capacity to ferment sugars which allowed them to colonize sugar-rich media over other yeasts, which were not tolerant to alcohol.

# Acetic Acid Bacteria (AAB)

The ninth edition of Bergey's Manual of Systematic Bacteriology classified the acetic acid bacteria (AAB) in the family Acetobacteriaceae and Gluconobacter. AAB are Gram-negative bacteria with ellipsoidal to rod-shaped morphologies. They are motile due to the presence of flagella, which could be either peritrichous or polar. They show a strict aerobic metabolism with oxygen as the terminal electron acceptor, and are catalase positive and oxidise negative.

Gullo and Giudici (2008) reported that AAB were present in the environment and in the raw material, but they ~~dear~~ cannot grow during alcoholic fermentation because of the anerobic conditions. when the alcoholic liquid was exposed to oxygen, the AAB started their growth on the surface. Most AAB grow between pH 5.4 to 6.3, but they also could grow at pH values lower than 4. They also reported that AAB could also be isolated at pH value of 2.0 to 2.3 in media containing acetate, if they were aerated. The optimal temperature for their

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growth was 25 - 30°C, but their growth was also observed between 35 - 40°C and weakly at temperature as low as 10°C

AAB were usually found in substrates containing sugar and ethanol. These substrates include fruits, flowers, food and fermented beverages, such as fruit juices, wine, cider, beer, cocoa and vinegar. The growth of AAB species isolated from fruits, flowers and fermentated foods are different from each other. They had displayed different abilities to grow using different culture media depending on the available nutrients. Results showed that there was a poor recovery on synthetic culture media due to the lack of suitable synthetic media, as not all synthetic media equally support the growth of AAB and could even be selective among strains.

Gracia - Garcia (2009) conducted studies on the acetous fermentation of ethanol into acetic acid by acetic acid bacteria belonging to the family Acetobacteriaceae and the genera Acetobacter and Gluconbacter. The total chemical reaction that took place was as follows

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$CH_3CH_2OH + O_2 \longrightarrow CH_3COOH + H_2O$

Ethanol Oxygen Acetic acid water

There were two steps in the oxidation of ethanol to acetic acid, driven by the enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH).

1st step was oxidation to acetaldehyde by ADH, which was further oxidised to acetic acid by ALDH. The reaction was exothermic, thus increased the temperature in the medium. The acetic acid could be further oxidised to carbon dioxide in the tricarboxylic cycle, that was an unwanted process in vinegar production. but it could occur when the ethanol concentration was limited and leads to the process, called over-oxidation, caused by bacteria belongings to Acetobacter because two of the key enzymes required for oxidation were non-functional in species of Gluconobacter.

Raspor and Goranovic (2008) reported that ethanol content affects AAB both in the beginning and at the end of fermentation. High initial ethanol concentrated decreased bacterial vitality

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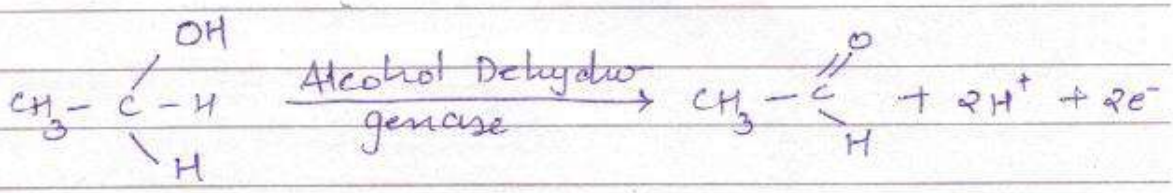
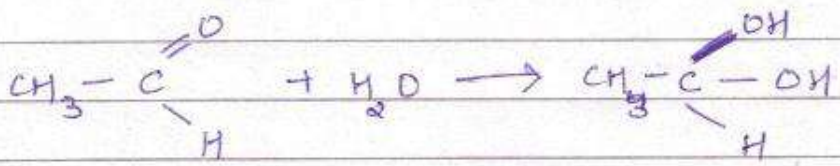
## ⇒ Chemical Reaction And Formulation

The production of acetic acid (Vinegar) is presented. Initially alcohol is hydrogenated to form acetaldehyde and two hydrogen ions and two electrons are released. In the second step two hydrogen ions bind with oxygen to form water that hydrates acetaldehyde to form aldehyde 3rd step - Aldehyde dehydrogenase converts acetaldehyde to acetic acid and releases 2 hydrogen ions and 2 electrons.

1. Formation of Acetaldehyde




2. Hydration of Acetaldehyde



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due to the antimicrobial effect of ethanol. And if instead, initial concentration was too low, (0.1 - 0.2 %) the risk for over-oxidation increased. When acetic acid concentration increased during fermentation, the pH decreased and reduced the bacterial activity, and set a limit for the concentration of acetic acid.

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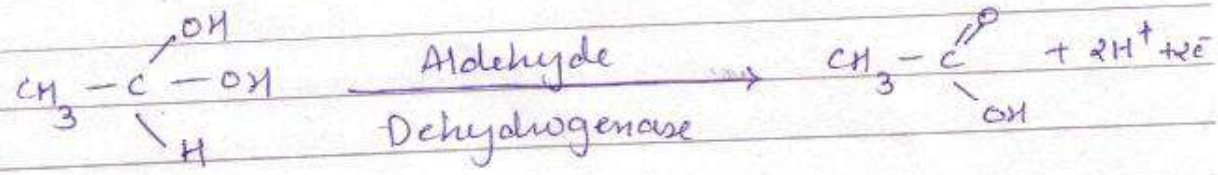
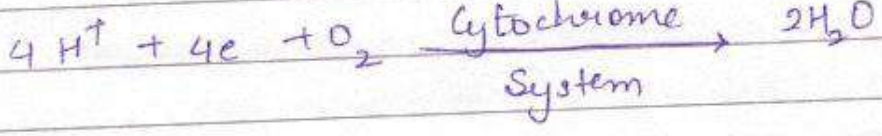
3. Formation of Acetic Acid




4. Electron Transfer



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## ⇒ Production Methods Of Vinegar

Vinegar production methods could range from traditional methods employing wooden casks (Orleans Process) and surface culture (Generator Process) to submerged fermentation. Vinegar is an important ingredient in many food products. The need for large amounts of the vinegar demands industrial fermentations systems that are capable of producing volumes that could reliably be controlled. Many technical devices have been developed to improve the industrial production of Vinegar. Generally these improvements increases the speed of the transformation of ethanol into acetic acid in the presence of AAB.

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Maazaand Murooka (2009) reported that the orleans process relies on the natural acetic acid bacteria present in the raw material, or makes the use of a seed cultures from a previous production batch. It was found that the bacteria, was belonging to the species Acetobacterxylium, and the growth on the liquid-air interface of the medium. Due to the oxygen requirement, the species were able to produce cellulose, a thick mat of a gelatinous substance containing bacterial cells and cellulose was developed over time on the top of the liquid. And the oxygen was moving into the mat used for the oxidation of ethanol into acetic acid. As a result the conc. gradient within the barrel was produced with a continuous diffusion of finish vinegar downward. The acetification process tooks a long time compared to more recent methods, with a production rate of about 1.°/. acetic acid per week.

Raspor and Goranovic (2008) The vinegar produced by this process was of high quality due to slow prodⁿ process that promotes the development of flavour and aroma. This process provides a constant availability of finished vinegar. But drawbacks is long time required hence high costs per volume.

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## • The Orleans Process

The slow method of acetifying wine which had been used in France since 1670 is known as the French or Orleans process. The Orleans process was the only method to make pure wine, vinegar and was reported to be the best process to produce fine quality table vinegar. In this process, wooden barrels were used and filled with alchol fermenting liquid to approximately 3/4 full which was provided with holes at the end of the barrel just a few inches abo the liquid surface. These holes were left open and covered with a fine screen.

Peppler and Beaman (1967) standardised the method of adding 20-25% of fresh vinegar into the barrel in order to acidify the liquid to the point of optimum growth for the vinegar bacte They reported that the bacteria settled into the liquid from the air and formed a gelatinous slime layer on the top of liquid. The liquid was then fermented for about 1 to 3 months at 70°F to 85°F.

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## • The Generator Process

In early 19th C, a vinegar making system called the trickle method now called generator process or quick process was developed by German scientist Schutzenbach in 1832. In this process, the bacteria were grown and formed a thick slime coating around a non-compacting material like beech wood shavings, charcoal or coke.

The fermentation was most commonly performed in tanks made of wood or steel. The surface area were where bacteria is exposed to oxygen was increased by a packing material in the tank, on which bateria were immobilised. The mostly used packing was made of beech wood shavings, over which the liquid was sprayed & then allowed to dip through the bottom to the reactor and the air was blown in from the lower part to maintain high oxygen availabili The partly finished vinegar that was accumulating at the bottom of tank was recirculated to the top again until the desired concentration of acetic acid was obtained. This process was performed at 27-30°C and a cooling coil in the tank was used to prevent overheating.

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There were some disadvantages associated with the generator process like high risk of clogging due to the cellulose-producing bacterial growth in the generator, accumulating of dead bacteria and infection with vinegar eels. Another disadvantage observed was a relative high loss of ethanol by evaporation that made it difficult to produce vinegar with high acetic acid ~~acor~~ concentration.

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- **SUBMERGED FERMENTATION**

The most common production method was submerged culture which improved the general fermentation conditions like aeration, stirring, heating etc. As generator culture was slow and expensive, submerged fermentors have been widely used at industrial scales. In this process, the mash is stired and aerated frequently and the fermentors are fitted with a heat exchange for the maintenance of the optimum temp. during the fermentation process. This is most common method used for commercial vinegar production where bacteria are suspended in medium, in contrast to traditional and generator process. The first bioreactor of submerged type was Fring's acetator in early 1950's. and is followed by other patented methods as cavitator, bubble column fermentor and Effgs turbine vinegator.

The fermentor was normally made of stainless steel with several different volumes. Basic principle was that the bacteria were free to substrate and air was forced into the medium by a stirer at bottoms of tank. The fermentor was equipped with a mechanical agitation which was generated fine bubbling in system. At the top of fermentor there

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was an air outflow, thermometer, cooling coils and a system to control and remove the build-up of foam. Studies revealed that system was very sensitive, since bacteria were dispersed in medium, even short interruption of air inflow and stirring could result in cell death.

Submerged process could be used for the prod" of vinegar in either a discontinuous, semi continuous or continuous system. In discontinuous system vinegar was produced in batches where a vol. of substrate was loaded and inoculated with bacteria and after acetification volume was completely unloaded from the fermentor.

Semi-continuous system was most commonly used & required a start-up period. Fermentors was loaded and inoculated. Once acetification had proceeded to desire level 40-50% of vol. was unloaded while the vinegar left behind was used as inoculum for next cycle. Advantage is shortened bacterial lag time for growth result in more efficient production.

Continuous system was base on constant composition of medium at a state where bacteria were in exponential growth phase & ∴ have their highest growth rate and produced a conversion of 8-9% acetic acid in 24-48 hours.

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## ⇒ PRODUCTION OF VINEGAR / PRODUCTIVITY OF VINEGAR

Vinegar sales grew at 15% from 2000 to 2002 and have been stronger than most other comparative categories including meat. Vinegar sales are somewhat seasonal, with a peak in summer months and secondary peak in april. There are some reports that suggest consumers are changing their vinegar purchasing habits.

Acc. of IRI 1998-2004 48% of households that purchase vinegar, 30% purchase distilled vinegar, 14% cider vinegar, 9% red wine, 5% balasmic vinegar, 3% rice vinegar.

Distilled vinegar remains strongest in sales, although white and ~~red~~ cidar are slowly increases in red wine,.

In 2005 blasmic vinegar made from grape had the largest ~~sell~~ sale with about 1/3 with cider vinegare share 7%.

In vinegar production, vinegar produced from other alcoholic base than wine, the acetic acid concentration threshold minimum is 5%.

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# ⇒ Types Of Vinegar

The predominant type of vinegar in US is white or distilled vinegar.
According to the Crisco Company, Vinegar varieties vary greatly from country to country. Some of the most popular vinegars and their characteristics.

1.) Balsamic Vinegar :- Brown in colour with sweet-sour flavour. Made from white Trebbiano grape and aged in barrels of various woods.

2.) Cane vinegar :- Made from fermented sugarcane and has very mild, rich sweet flavour. It is most common in Phillippine cooking.

3.) Champagne Vinegar :- It is made from still, dry, white wine. Made from Chardonnay or Pinot Noir grapes.

4.) Cider Vinegar :- Made from apples and is most popular vinegar used for cooking in US.

5.) Coconut Vinegar :- Low in acidity with a musty flavour and a ~~unique~~ unique taste. It is used in Thai dishes.

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6.) Distilled Vinegar :- Harsh vinegar made from grains and usually colourless. Best used for pickling.

7.) Malt Vinegar :- Popular in England. Made from fermented barley and grain mash, and flavoured with woods such as beech or birch. Hearty flavour and is often served with fish and chips.

8.) Rice wine Vinegar :- Made by chinese for over 5000 yrs. 3 kinds of rice wine vinegar, red (used as dip for foods and as a condiment in soup) white (used mostly in sweet and sour dish) and black (common in stir-fries and dressing).

9.) White vinegar :- Made from distilled vinegar. The term "distilled" is misleading, because vinegar is not distilled but it is made from distilled alcohol.

10.) Wine or Grape Vinegar :- Made from grapes by acetic fermentation is called "Wine" or "Grape Vinegar". It can be made from red, white or rose wine. The vinegars is makes the best salad dressings.

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## ⇒ USES OF VINEGAR

The use of vinegar to flavour food is centuries old. whether naturally produced during fermentation or intentionally added, vinegar retards microbial growth, and contributes to no. of foods. It has been used as medicine, corrosive agent, painkilling agent and can be directly consumed in diluted form as a beverage. In the food industry, vinegar is used mainly as an acidulant, but it has also many other food processing applications. It is found in food including salads dressings, mayonnaise, mustard, ketchup, bread and bakery products, canned food, marinades and the current falling wine consumption have favoured an increase in vinegar production.

Vinegar is the world's oldest cooking ingredients and food preservation method.

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## ⇒ VINEGAR QUALITY

The vinegar quality depend on process conditions including acetification speed. The rate of fermentation influences the sensory propeties of the final vinegar but some believed there were no differences between vinegars obtained at different fermentation speeds.

At least two criterians were kept in consideration to assess the quality of vinegar. The first criterions was based on establishing that vinegar is actually vinegar not diluted acetic acid. Discriminating analyses that were used to distinguish samples of vinegar involved measuring selected chemical constituents, including minerals, alcohols, acids, phenols, & other volatile compounds. Using such an approach it was even possible to differentiate fermentations proces used to produce vinegar.

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## ⇒ VINEGAR AROMA

The characterization of vinegar include a wide range of values obtained from physio-chemical and sensory parameters. ~~Various~~

The other main characteristics on which vinegar quality was based were those that related to flavour, aroma and other organoleptic properties. Vinegar flavoured was particularly influenced by the raw ethanolic material from which it was made. And although acetic acid was by far the predominant flavour present in vinegar, other volatile flavour compounds were also present that contributed to overall flavour profile of vinegar. In addition to this, the flavours and aroma were dependent on the method of prowss, aging time and raw material used in the mash.

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## ⇒ HEALTH ASPECTS

Phenolic compounds present in apples could prevent diffuent chronic disorders such as cancers and cardiovascular diseases. Vinegar is used in food preparation and also used to treat wounds and infections. It could also be used in food preservation due to this low pH that retarded microbial growth and contributed sensory properties to a number of foods.

Vinegar possessed an antiglycemic effect and decreased the glycemic index in a meal that could provide health benefits in both healthy and in diabetic patients. Vinegar could be used to bear on wounds, ulcers, arthritis, high blood sugar, high cholestirol, urinary tract and yeast infections, sun burn, insects bites, warts and hiccups.