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Manufacturing of Cost Effective Vinegar from Different Fruit Products by Acetobacteria

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Abstract: *Vinegar may be defined as a condiment made from various sugary by alcoholic and subsequent acetic fermentation. The vinegar bacteria, also called acetic acid bacteria. Aceto acetic bacteria having ability to convert ethyl alcohol (C_2H_5OH) into acetic acid (CH_3COOH) by oxidation. Vinegar can be produced from various raw materials like Apple cider, Honey, Sugarcane, Pomegranate, Coconut, Orange peel, Grapes, Tomato, Rice by several major production techniques for making vinegar such as the Orleans process, generator process and Submerged acetification process. The generator process is Non compacting material is filled in the large upright wood tanks above a perforated wood grating floor. Re-circulated fermenting liquid trickles over packing material toward the bottom while air moves from the bottom inlets toward the top. The recirculation process takes about 3 to 7 days after which 2/3 of the final vinegar product is withdrawn from the tank and new alcohol solution is added. Submerged acetification in this process supply air is forced into the alcohol liquid in the tank and the material is fermented at 30°C. At the end of every cycle, 1/3 of the liquid is discharged as final product, replaced with mash containing fresh alcohol solution and a new fermentation cycle begins.The Gram stain method can be used to classify gram-positive or gram-negative bacteria. Gram staining can narrow down the identity of vinegar cultures to gram-positive and negative classes, and then the cultures can be identified to a specific species by using the polymerase chain reaction (PCR). A pink colour demonstrates gram-negative character and a blue colour indicates gram-positive. Vinegar cultures are predominantly gram-negative bacteria.*
The aim in the present study is to identify quality and %concentration of acetic acid in the fruit product and to characterize the species of vinegar bacteria used in acetification.

1. Introduction

Vinegar is product from the conversion of Alcohol to acetic acid by bacteria. VIN=Wine; gar=sour. When alcoholic fermentation occurs and later during acidification many other compounds are produced.Vinegar is a liquid consisting of about 5–20% acetic acid (CH_3COOH), water, and other trace chemicals, which may include flavorings. The acetic acid is produced by the fermentation of ethanol by acetic acid bacteria. Vinegar is now mainly used as a cooking ingredient, or in pickling. Historically, as the most easily available mild acid, it had a great variety of industrial, medical, and domestic uses, some of which (such as its use as a general household cleanser) are still practiced today.Commercial vinegar is produced either by fast or slow fermentation processes. In general, slow methods are used with traditional vinegars, and fermentation proceeds slowly over the course of months or a year. The longer fermentation period allows for the accumulation of a nontoxic slime composed of acetic acid bacteria. Fast methods add mother of vinegar (bacterial culture) to the source liquid before adding air to oxygenate and promote the fastest fermentation. In fast production processes, vinegar may be produced in 20 hours to three days. [1]

Vinegar may be defined as a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation .Vinegar can be produced by different methods and from various raw materials. Wine (white, red, and sherry wine), cider, fruit musts, malted barley, or pure alcohol are used as substrates. Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation in acetators. Vinegar traditionally has been used as a food preservative. Whether naturally produced during fermentation or intentionally added, vinegar retards microbial growth and contributes sensory properties to a number of foods. The wide diversity of products containing vinegar (sauces, ketchup, mayonnaise, etc.) and the current fall in wine consumption have flavoured an increase in vinegar production. Acetic acid is the predominant flavouring and antimicrobial component in vinegar. The following review will focus on the importance of acetic acid as a direct food additive or more recently as a food processing aid, to decontaminate food prior to distribution and consumption. Earlier processes used for making vinegar were the Orleans process (which is also known as the slow process), the quick process (which is also called the generator process), and the submerged culture process. The quick process and submerged culture process were developed and are used for commercial vinegar production today. [2]

Acetic acid is formed in a four-step reaction involving conversion of starch to sugar by amylases, anaerobic conversion of sugars to ethanol by yeast fermentation, conversion of ethanol to hydrated acetaldehyde, and dehydrogenation to acetic acid by aldehyde dehydrogenase. The last two steps are performed aerobically with the aid of acetic acid forming bacteria. Acetic acid yield from fermented sugar is approximately 40%, with the remaining sugar metabolites either lost to volatilization or converted into other compounds. Acid yield improvements can be achieved using high rates aeration of during continuous production. Vinegar bacteria, also called acetic acid bacteria, are members of the genus Acetobacter and characterized by their ability to convert ethyl alcohol into acetic acid by oxidation Most bacteria strains derived from vinegar factories are able to oxidize acetic acid to CO2 and H2O (over-oxidation) and therefore are classified in the genus Acetobacter.Common types of vinegar include white distilled vinegar, cider vinegar, wine vinegar, rice vinegar, and malt vinegar. Further processing of vinegar, following substrate conversion to acetic acid may include filtration, clarification distillation and pasteurization at 165.2°F (74°C) before it is bottled. Regulations in the United States require vinegar to contain at least 4% acetic acid resulting from acetic acid fermentation of ethanol containing substrates. Labels identifying the diluents used to meet the listed concentration of acid are also required. Acetic acid concentration in vinegar may be expressed using the term "grain". For example, 100 grain distilled vinegar is a 10% acetic acid solution. If higher concentration of acetic acid is required, the dilute solution of acetic acid maybe heat distilled or frozen to slush. The slush is centrifuged to isolate the liquid portion. Concentration from 10-30% may be achieved using this technique.

Vinegar plays an important role in salad dressings, ketchup, hot sauce and other sauces. This need demands industrial fermentation systems capable of producing a large amount of vinegar. These systems must maintain reliable controls and optimum conditions for acetic acid bacteria fermentation. Many techniques have been developed to improve industrial production of vinegar. Most try to increase the speed of the transformation of ethanol into acetic acid in the presence of the acetic acid bacteria. Today, the most common technology for the vinegar industry is based on the submerged culture with diverse technical modifications which try to improve the general fermentation conditions (aeration, stirring, heating, etc.). Specific goals were to achieve 8-10% acidity using constructed lab scale production facilities and to characterize the species of vinegar bacteria used in acidification. [3]

Vinegar contents: Apple Cider, Sugarcane, Coconut, Orange Peel, Grapes, Tomato, Honey, Rice, Pomegranate

The formation of vinegar: Acetic acid bacteria are well known for their ability to spoil fruits because they can produce large amounts of acetic acid from ethanol and other compounds present in fruits.

Uses: Food condiment, Treatment of wounds, Cleansing agent, Cosmetic Aid, Flavouring for food products, Food Condiment, sprinkled on certain food such as fish at the table, Preservatives of pickling, meats, vegetables etc., Vinegar has been reputed to have strong antibacterial properties, 20% acetic acid vinegar can be used as an herbicide

2. Method:

Vinegar production methods can range from traditional methods employing wood 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 fermentation systems that are capable of producing volumes that are reliably controlled. Many technical devices have been developed to improve the industrial production of vinegar. Generally, these improvements increase the speed of the transformation of ethanol into acetic acid in the presence of acetic acid bacteria.

Vinegar qualities characteristics:

The vinegar qualities depend on process conditions including acetification speed. The rate of fermentation influences the sensory properties of the final vinegar, but some believe there are no differences between vinegars obtained at different fermentation speeds. Experts usually detect important sensory differences between vinegar manufactured by the submerged and generator processes.

Market Survey:

Year	Existing production	Unsatisfied Demand
2013	50,750	55,074
2014	51,511	58,546
2015	52,284	62,175
2016	53,068	65,969
2017	53,864	69,934
2018	54,672	74,073
2019	55,492	78,403
2020	56,324	82,927
2021	57,169	87,652

Table 1 Import of vinegar by India (2001-2010) (The Vinegar Institute)

Year	Import (liters)
2001	13476
2002	31392
2003	26008
2004	21458
2005	28990
2006	33879
2007	47082
2008	45507
2009	54524
2010	81832

Table 2 Projected demand for vinegar in liters (The Vinegar Institute)

Subculture procedure for Acetobacter:
Apparatus required: Conical flask, Nichrome loop, Acetobacter culture, Air filter, Autoclave.
Chemical required: Tryptone powder, Yeast extract, Glucose powder, Calcium carbonate.
Study procedure and data collection procedure: It is necessary to sterilize the conical flask for which it is to be kept in auto cave for about 12 hrs. Take 100gm of sample in a glass flask after autoclaving in conical 400ml of distilled water is to be added. Then add Tryptone powder and yeast extract to flask in proportion of 12gm for every 100ml of distilled water it is required to add 8gm of Tryptone powder and 4gm of yeast extract. (For our process we require 400ml of bacterial suspension per batch)Using Nichrome loop take loop full of bacteria from culture tube and transfer it in flask carefully. This transfer process has to be carried out under heap air filter in order to maintain purity of bacteria. At last add glucose and calcium carbonate to prepared solution with same proportion as taken for Tryptone powder and yeast extract .Keep suspension solution undisturbed for next 24 hrs. This solution then can be viewed under microscope to ensure the growth of bacteria (While carrying out every single step utmost has to take of maintaining purity of culture tube and bacterial suspension).This prepared solution can be including beech wood having in generator strength calculation of project. For strength estimation of vinegar simple procedure of titration can be followed. Take 10ml of product sample in conical flask ad 2-3 drops of

phenolphthalein indicator to it .Rinse and fill the burette with 0.1N NaOH. Titrate of flask against burette solution .Note the volume of NaOH consumed. Follow same procedure for different time.

Gram Stain: The Gram stain method can be used to classify gram-positive or gram-negative bacteria. Gram staining can narrow down the identity of vinegar cultures to gram-positive and negative classes, and then the cultures can be identified to a specific species by using the polymerase chain reaction (PCR). For the Gram stain, 1ml of culture sample was placed into a 1.5ml Eppend tube and centrifuged 12gm. for 8 min. A drop (approximate 0.18 gram) of the bacteria culture sample was removed from the tube, smeared on a slide, and allowed to dry. After drying, the bacteria were heat fixed to the slide. Crystal violet pigment was added to the smear for 1 minute. After 1 minute, the pigment was washed off with distilled water. Then iodine was applied for 1 minute. The iodine was Water Bath Thermometer Nichrome loop Holder and washed off with distilled water again. The smear was decolorized with 95% ethyl alcohol for 3 seconds. The alcohol was removed with distilled water and the smear was counterstained with safranin for 1 minute. The safranin was removed with distilled water and the slide dried with a paper towel. After drying, the slide was mounted under a microscope (Optics, IL) with 10X100 magnification. A pink colour

Demonstrates gram-negative character and a blue colour indicates gram-positive. Vinegar cultures are predominantly gram-negative bacteria.

Name of fruit products:	Colour	Result	Conclusion
Apple cider	Pink	gram-negative	Vinegar is present
Sugarcane	Pale pink	gram-negative	Vinegar is present
Coconut	Pale pink	gram-negative	Vinegar is present
Grapes	Pale pink	gram-negative	Vinegar is present
Tomato	lighter blue	gram-positive	Vinegar is may be present
Pomegranate	Pink	gram-negative	Vinegar is present

The pain perception and Gram strain techniques result were shown in *Table3*. It includes presence of Acetobacter culture.

Level of statistical meaning fullness (Table3).A pink colour demonstrates gram-negative character and a blue colour indicates gram-positive. Vinegar cultures are predominantly gram-negative bacteria.

Tomato may not be containacetobacterial culture.

In the Apple cider for first three days acidic concentration increase slowly later in day 4th, 5th, 6th sudden increase in acidic concentration of apple cider then constant percentage is arrived, at 8th day %concentration of acetic acid in apple cider is 6.2023 %.(Table 4)

In sugarcane acetic acid contain is constant after 8^{th} day and at the day 8^{th} is 4.3691. (Table 4)

In the Coconut for first three days acidic concentration increase slowly later in day 4^{th}, 5^{th}, 6th, 7th sudden increase in acidic concentration of Coconut then constant percentage is arrived, at8^{th} day %concentration of acetic acid in coconut is 3.319%. (Table 4)

In Grapes acetic acid contain is constantly increase and at the day 8^{th} is 4.46242 %.(Table 4)

In the pomegranate for first three days acidic concentration increase slowly later in day 4^{th}, 5th, 6th sudden increase in acidic concentration of pomegranate then constant percentage is arrived, at 8th day %concentration of acetic acid in pomegranate is6.3815%.(Table 4)

DAY-8 Normality of NaOH-1 N

Name of fruit products	C.B.R.	Normality Of vinegar	Concentration (gm/lit)	(Concentration/density of vinegar)	%concentration
Apple cider	10.73	1.073	64.38	62.023	6.2023
Sugarcane	7.56	0.756	45.36	43.691	4.3691
Coconut	5.742	0.5742	34.45	33.19	3.319
Grapes	7.72	0.772	46.32	44.6242	4.46242
Tomato	6.183	0.6183	37.098	35.739	3.5739
pomegranate	11.04	1.104	66.24	63.815	6.3815

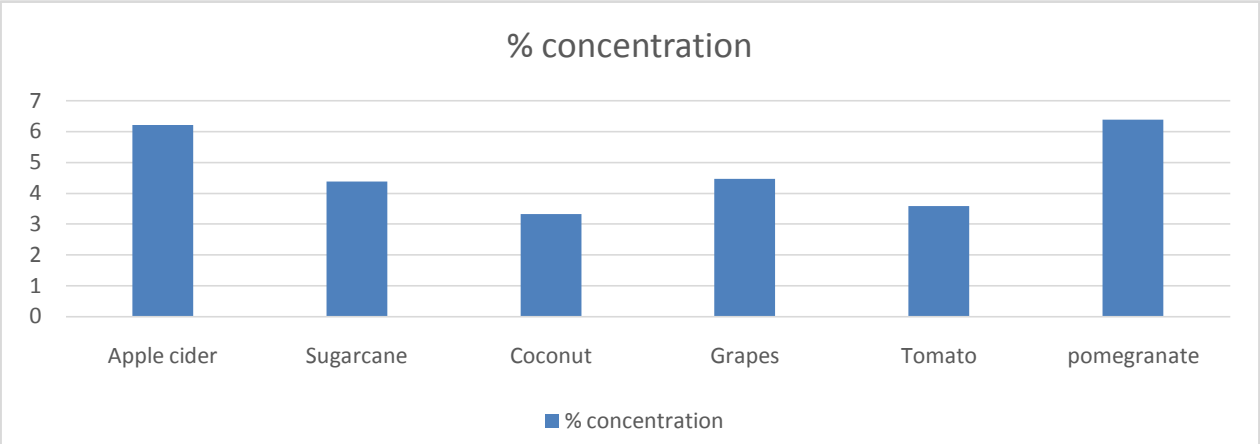

Table 3 Result of gram strain

4. Discussion:

The influence of vinegar has been investigated for hundreds of years. In fact it was first used about 5000 years ago. In the year 400 B.C., Hippocrates, the father of modern medicine, prescribed the mixture of honey and apple cider vinegar for treatment of various diseases. It has been particularly used during the American Civil War for dis infecting the wounds of oldiers Vinegar is produced from fruit juice such as grape, apple, coconut, and tomato, rice. It is made by crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid to start the alcoholic fermentation process, and the sugars are turned into alcohol. In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming bacteria (Acetobacter) Vinegar is a plant based product has been known and used for unknown years. Vinegar has many proven positive effects on health such as an antibacterial effect, reduct ionic blood pressure, an antioxidant effect, an anti-diabetic effect, an antitumor effect, reduction and prevention of obesity, an antihypertensive and cholesterol decreasing effect, a healing effect on injuries, and appositive effect on brain and cognitive functions. Apple products are widely used in the world.

The submerged pilot unit produced vinegar with an acid strength of 6.3815 % in 8days from pomegranate. This was a slow process to produce vinegar and it is cost efficient. In addition, it appears that there was a loss of alcohol under this process possibly because the surface area was so large. The bacteria in the generator were slow growing even when the generator unit was operating under perfect conditions. It took 5 days to start this generator but sometimes as much as 10 days are needed to start a unit under perfect conditions.

This method minimizes the alcohol and acetic acid loss. The bacteria will grow easily in the aerated liquid under perfect conditions. The submerged process was easy to start compared to the generator process.

Many people believe the submerged and generator processes give different flavours to the vinegars. The differences might be due to the beech wood shavings as indicated by the submerged test with beech wood powder. The beech wood may impart flavours, just like aging of whiskey in the oak barrels.

Gram staining indicated that the predominant bacteria in all studied processes were gram-negative bacteria as it should be the submerged acetification bacteria.

5. Conclusion:

Vinegar plays an important role in salad dressings, ketchup, hot sauce and other sauces and it also had some medicinal uses also. Vinegar need demands industrial fermentation systems capable of producing a large amount of vinegar. These systems must maintain reliable controls and optimum conditions for acetic acid bacteria fermentation. Pomegranate, apple cider are the main source of aceto-bacteria. It contain more than 6% acid concentration.
Our techniques have been developed to improve industrial production of vinegar. Most try to increase the speed of the transformation of ethanol into acetic acid in the presence of the acetic acid bacteria.

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