Study on Production Vinegar from Apple

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Abstract. The goal of the present study was to ascertain the effect of the addition of certain natural materials such as sugar or Jaggery Syrup, Black Gram, or extracts there of as fortified materials on the consistency of apple vinegar and the types of microorganisms in it. The findings revealed that the addition of whole legume or their extracts during processing enhanced the properties of the resulting vinegar. The findings showed that the better pH was for apple vinegar produced with the inclusion of sugar and protein extract, which had a pH of 3.1. This was accompanied by vinegar resulting from the addition of jaggery with whole legume, where the pH was 3.8, followed by vinegar resulting from the addition of whole legume plus sugar, where the pH was 3.9. Though apple vinegar obtained by adding protein extract with jaggery had a pH of 4. As it was influenced by the acidity of the resultant vinegar due to the content of legumes and sugary compounds, the maximum acidity was 6.5 in the vinegar to which entire legume jaggery was applied. Then vinegar, to which whole legume sugar was added, and vinegar, to which jaggery with protein extract was added, the acidity reached 3. Finally, vinegar made by combining sugar with protein extract, where the acidity reached 2. The best types of vinegar were selected from among the samples (vinegar developed by the addition of protein extract jaggary) and the contents of its microbiology were analysed. The results showed that the vinegar was free of bacteria and yeasts, while its content of moulds was 10³/g. The four samples of vinegar also showed good antioxidant activity. It was 86.77%, 85.38%, 79.0% and 19.11% in vinegar containing jaggery with extract protein, sugar with extract protein, jaggery with whole legume and sugar with whole legume respectively. The total solde of vinegar were different, 4.8%, 4.5%, 4.0%, and 3.0% in vinegar containing jaggery with whole legume, jaggery with extract protein, sugar with whole legume and sugar with extract protein. The colour analysis of the vinegar sample containing the jaggery with extract protein was 75.91, 4.87, 51.4 for the lighter side, red colour and greener side, respectively.

1. Introduction
By alcoholic fermentation and subsequent acetic fermentation[1], vinegar can be represented as a condiment made from various sugar and starchy materials. It is possible to manufacture vinegar using different processes and different raw materials. Wine (white, red and sherry wine), beer, fruit musts, malted barley, hydrolysed cereals and starches, or pure alcohol can contain substrates. It is formed as a liquid formed by yeast and acetic acid bacteria to ferment ethanol in a process that generates the main ingredient, acetic acid. Concentrations of acetic acid typically range from 4% to 8% by volume for table vinegar (usually 5%) and higher concentrations for pickling (up to 18 percent). Reduced amounts of tartaric acid, citric acid and other Acids are also found in natural vinegar. Vinegar has historically been used as a food preservative, The vinegar, whether spontaneously developed through fermentation or intentionally added, prevents microbial growth and adds sensory properties to a number of foods. The vast variety of vinegar-containing products (sauces, ketchup, mayonnaise, etc.) and the recent decline in the consumption of wine have favored an increase in the market for vinegar[2] The main flavouring and antimicrobial component of vinegar is acetate. Vinegar production ranges from traditional methods of wood casks and surface cultivation to submerged fermentation in acetates[3] The Orleans process (also known as the slow process), the quick process (also known as the generator process) and the submerged culture step were previous techniques used to manufacture vinegar. Vinegar production ranges from traditional methods of wood casks and surface cultivation to submerged fermentation in acetates[3] The
Orleans process (also known as the slow process), the quick process (also known as the generator process) and the submerged step of cultivation were previous techniques used to manufacture vinegar. The rapid method and the submerged process of cultivation have been developed and are commonly used for the industrial production of vinegar. For traditional types of vinegar, sluggish methods are usually used and fermentation takes place slowly over weeks or months. The longer fermentation time causes the accumulation, known as the mother of vinegar, of a non-toxic slime made up of acetic acid bacteria and soluble cellulose. Fast methods apply mother vinegar (i.e. bacterial culture) to the liquid source and then use a venturi pumping machine or turbine to add air to facilitate oxygenation to achieve the fastest fermentation. Vinegar can be produced over a period of between 20 hours and three days using quick processing methods.

2. Materials and Methods
The samples and the ingredients were bought from local fruit shops and grocery stores. Chennai –India.

2.1. Ingredients Needed For The Production Of Vinegar
Water 250 mL / 500 mL of vinegar. Salt - 0.645g / 500 mL of vinegar. Sugar/Jaggery Syrup(made from 50g in 200 mL of water)/ 500 mL of vinegar. Starter Culture(Yeast) – 0.75g/500mL of vinegar .legume or extract protein 1g or 1mL / 500 mL of vinegar.

2.2. Protein sours:
Protein was added with the fermentation broth from black gram given below either whole or the extract of the pulse made with cold water. Whole legume and extract -1g or 1 mL Black gram .The process for vinegar fermentation consisted of three major steps as given below. Preparation of Mash. Alcoholic fermentation .Acetic acid fermentation.

As defined, the titratable acidity (TA) was calculated. [4] [5] mL of 1N NaOH was used to achieve a pink phenolphthalein colour endpoint. Dry phenolphthalein (0.002 grams) was applied to each 10 mL vinegar solution sample. The primary organic acid in vinegar is acetic acid. The formula for the acetic acid percent TA estimate is as follows.

\[
\%TA = \frac{(mL \ of \ NaOH) \times (N \ of \ NaOH) \times (60.05)}{10 \times \text{Sample Weight}}
\]

Antioxidant Activity was determined according the method described by [6]

2.4. DPPH ASSAY
A methanol solution of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared. They prepared the extracts by dissolving them in methanol. 190 μL of DPPH solution was applied to the microtitrate plate and 10 μL of extracts were added to it and incubated at room temperature for 30 minutes in dark conditions. After the incubation period, the absorbance was measured at 517 nm. For the control well, 10 μL of extract was replaced by 10 μL of methanol. The DPPH scavenging activity was calculated by the following formula:

\[
\text{DPPH scavenging activity (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100
\]

2.5. Quantification Of Microbes
Procedure for plate counting: Weigh 10 μL of sample into 90 mL of saline in a test tube. This gives. 10-5the sample dilution. To allow blending, shake gently. Pass 0.2 mL of the sample to a plate of sterile nutrient agar and distributed thinly using a sterilized, cooled glass spreader of alcohol. For 24 hours, incubate the plates at 37oC. Count the number of bacteria present in the specified sample as CFU /mL/g. The number of bacteria deterred in compliance with the system mentioned in [7].

2.6. Identification Of Bacteria
The Test was made to identification the bacteria in sample [8].

2.7. Detection Of Antibacterial Activit
In order to establish the antibacterial activities of isolates, the identification of the antimicrobial activity of vinegar using the agar-well diffusion method mentioned by [9] with minor modification was used. Indicator bacteria were incubated at 37ºC for 18 h (Table 1). 10 mL of LB-Agar with 1% agar was poured into each 100 mm petriplates and allowed to solidify. The indicator bacteria were aseptically swabbed on surface of LB- Agar using sterile cotton swab. Once the Agar was solidified, it was punched with a six millimeters diameter wells and filled with 100µl of cell free supernatant of 24 hours fresh grown isolate obtained by centrifuging the culture broth at 5000 rpm for 15 min. Plates was incubated at 37ºC for 24 hours the clearance zone was measured in millimetre.

| NO. | Bacteria of the Measure  |
|-----|--------------------------|
| 1   | Bacillus subtilis         |
| 2   | E coli                   |
| 3   | Micrococcus luteus       |

2.8. Assessment Of Antibacterial Susceptibility

The disk diffusion method described by was used to determine the susceptibility or resistance of the isolates (bacterial strain) to an antibacterial agent (antibiotics).10 mL of MRS broth with 1% agar was poured into each 100 mm petri plates and allowed to solidify. 100µl of (isolates) was aseptically swabbed on surface of MRS-Agar using sterile cotton- swab and after solidification four different antibiotic discs were kept in four quadrants in each plate, and incubated the plate at 35ºC for 24- 48 hours. The zone of inhibition was measured in millimetres. In compliance with the guidelines published by[10] , strains were graded as resistant (R), susceptible (S) or intermediate (I) .

2.9. Physical Test

2.9.1. Determination Of Total Solids Using Refractometer

The speed at which light travels will vary based on the parameters of the materials as light moves from one medium to another. When looking at a straw in a glass or an oarsman on the boat, the theory can be seen. The ratio or difference in the speed of light is called refractive index & refractometers are called instruments that calculate this parameter. A liquid's refractive index is related to its concentration and so the concentration can be displayed in relevant units such as brix by a refractometer ( percent sugar) [11]. the experiment was conducted according to the method described in. Refractometers are instruments to measure substances dissolved in water & certain the brix value is arguable & the most important parameter in quality control [12].

2.9.2. Colour Analysis

The Lab colour space mathematically defines all perceivable colours for lightness in the three dimensions L* and green-red and blue-yellow for the colour adversaries a* and b*. For device-independent digital representation, space is typically mapped to a three-dimensional integer space, and for these purposes, the L*, a*, and b* values are usually absolute, with a pre-defined set. The shine, L*, is the darkest black at L* = 0, and the brightest white at L* = 100. The a* and b* color channels reflect true neutral gray values at a* = 0 and b* = 0. Along the* axis, the red/green adversary colors are depicted, with green at negative values a* and red at positive values a*.Along the b* line, the yellow/blue opponent colors are seen, with blue at negative b* values and yellow at positive b* values. Using Hunter Lab's tool, the color analysis was performed. [13]; [1]

3. Results and Discussion

3.1. Product Obtained

After 40 days of fermentation at room temperature, vinegar from different sources was obtained. The vinegar samples obtained were coloured based on the raw material and the ingredients used. The samples obtained from Apple were brightly coloured with red hues owing to the colour of their source material whereas the samples irrespective of the source material, with jaggery tend to show yellowish colour.
3.2.1 Quality Assessment

Measurement Of PH

Figure 1 showed that PH of vinegar was made from apple was 2-3 while the PH of vinegar sugar with whole legume was 3.9 and vinegar made from apple with jaggery with whole legume 3.85. PH of vinegar with sugar with extract protein 3.15 and the vinegar with sugar and extract protein 4.

![Figure 1. pH values of different vinegars](image1)

The USDA standard (U.S. Department Of Agriculture) value for pH has to be in the range 3.15 to 3.9, below which the vinegar tends to become toxic and above which the value indicates that the vinegar produced is not completely dehydrogenated to acetic acid and the reaction is not yet complete. All of the samples obtained were within the range.

Acetic Acid Analysis

According to FDA (U.S. Food and Drug Administration), the vinegar should contain acetic acid no less than 4%. Most of the samples obtained are well between the range 4% to 9% with some exceptions (Figure 2). Whole legume protein with sugar 3 protein extracted with sugar 2, extract protein with jaggery 3. Whole legume with jaggery 6.5.

![Figure 2. Acidity values of different vinegars](image2)

The formation of less acetic acid (4%) can be due to the improper conversion of ethanol to acetaldehyde or unavailability of microorganisms involved in the mechanism of fermentation. There is not much difference in the acidity values in all the samples when compared between sugar and jaggery.
But in the comparison between whole protein and protein extract, the samples with whole protein have higher values for acetic acid which is due to the presence of other nutrients like carbohydrates, fats, etc in the whole protein unlike the protein extract which contains only protein.

**Antioxidant Activity**

Antioxidants are a class of molecules capable of inhibiting another molecule's oxidation. Antioxidants play an important role in your wellbeing, and by combating free radicals that result in oxidative stress, they can control how quickly you mature. Due to their strong antioxidant effects, polyphenols and vitamins in several various forms of vinegar support avoid oxidative stress. For example, catechin, epicatechin, and gallic, caffeic, and chlorogenic acids are some examples of antioxidants in apple cider vinegar (Table 2).

**Table 2. Antioxidant activity of different vinegars**

| Sample | % inhibition activity |
|--------|-----------------------|
| jaggery with whole legume | 79 |
| sugar with whole legume | 19.11 |
| sugar with protein extract | 85.83 |
| jaggery with protein extract and | 86.77 |

The % inhibition activity calculated from the absorbance values were higher than 60 % for all the samples denoting that the antioxidant activity is good in all the samples irrespective of production from various raw materials and the variations. When compared between sugar and jaggery, the samples with jaggery had higher % of inhibition activity. Likewise, when compared between whole protein and extract protein, both had nearly similar values of % inhibition.

**Antibacterial Activity Of Vinegars**

antibacterial behaviour in vinegars. The quality of acetic acid and the pH value may be related. The antimicrobial effectiveness was measured against separate bacteria for both vinegar and acetic acid. Entry includes the process of microbial inactivation by organic acids, including acetic acid, An un-dissociated form of organic acid (HA) via the cell membrane, in combination with intracellular pH and dissociation into (H+) and (A-) ions. In order to improve the corrosiveness and increase the sharpness of the cytoplasm, Proton triggers cell damage and modifications or denaturation of catalysts and primary proteins, such as ruining DNA/RNA amalgamation, The presence of phenolic compounds in vinegar is not only responsible for its effective cancer prevention agent, but also limiting the cell to use the findings. Vinegar's antimicrobial exercises are also identified for their acidic corrosive content and pH principles. The results of this current research uphold and reinforce the evidence on the possibilities of different vinegar forms relevant to well-being advancement and food handling, and how these properties are impacted by different preparation techniques that can be used for creation .

**Table 3. The pH and overall acidity values and the diameters of the various vinegar inhibition zones**

| PH, Acidity and Inhibition Zone Interaction | sugar with whole legume | jaggery with whole legume | sugar with extract protein | sugar with extract protein |
|-------------------------------------------|-------------------------|---------------------------|---------------------------|----------------------------|
| PH | 3.9 | 3.8 | 3.1 | 4 |
| Acidity | 3 | 2 | 3 | 6.5 |
| Bacillus subtilis(mm) | 11.5 ± 2 | 10.8±2 | 11.2 ±2 | 9.7 ±2 |
| E coli(mm) | 10.4 ± 2 | 9.6±2 | 10.3 ±2 | 8.3 ±2 |
| Micrococcus luteus(mm) | 11.2 ± 2 | 10.9±2 | 11.6 ±2 | 9.7 ±2 |
Total Soluble Solids
The Brix values obtained for all the samples were in the range of 3 to 4.5 denoting that the viscosity of the samples are less. The least value is for with 3 and highest for coconut with 4.5 (Figure 3).

The Brix values obtained for all the samples were in the range of 2.5 to 7 denoting that the viscosity of the samples are less. The least value is for pomegranate with 2.7 and highest for coconut with 6.8 (Figure).

Colour Analysis
The table (4) shows the L* values for all the samples were on the brighter side. The a* values were mostly positive denoting that those were having red colour. The b* values obtained for all the samples were positive showing that the colour of all the samples were to the greener side.

| Colour analysis | L*   | a*   | b*   |
|-----------------|------|------|------|
| Apple           | 75.91| 4.87 | 51.4 |

Microbial Plate Counting
The results in table show that all samples had a good proportion of microbial growth with some samples having all the three microbes: bacteria, yeast and mould. The samples with no yeast, mould denoted possibly that there is complete conversion of alcohol and the domination of acetic acid bacteria. Similarly, the plates that had more yeast development showed the yeast is already left for the conversion of ethanol sugars.

| Sample                        | BACTERIA (X 10^5 CFU/ML) | YEAST (X 10^5 CFU/ML) | MOULD (X 10^5 CFU/ML) |
|-------------------------------|--------------------------|-----------------------|-----------------------|
| with sugar and whole protein  | 1                        | 0                     | 3                     |
| with jaggery and extract      | 2                        | 3                     | 2                     |
| protein                       |                          |                       |                       |
| with sugar and extract protein| 0                        | 0                     | 4                     |
| with jaggery and whole        | 2                        | 2                     | 2                     |
| protein                       |                          |                       |                       |
4. Conclusion
In this analysis, local apple vinegar was examined by conducting different tests such as acetic acid content, pH, total soluble solids, anti-oxidant activity, antimicrobial, and other physicochemical tests. Anti-microbial tests were also carried out but the results were inconsistent. Two variations of vinegar were made with extract protein and sugar, whole protein and sugar and whole protein with jaggery and extract protein with jaggery. The latter was stored for tasting and the three other variations were tested in labs. It was found that the variation having jaggery and whole protein yielded more in case of microbial count, pH, total soluble solids and anti-oxidant, sugar & whole protein in case of acetic acid. It was found that the variation having jaggery and whole protein yielded more in case of microbial count, pH, total soluble solids and anti-oxidant, sugar & whole protein in case of acetic acid. Use this processed industrially will give us a product with a good economical value. Further studies needs to be done on the product to stabilise its sustenance in the ever growing market.

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