

ORIGINAL ARTICLE

Natural Resource Utilization by Acetic Acid Fermentation: A Method for Post Harvest Management

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ABSTRACT

Acetic acid (commercially known as "vinegar"), one of the culinary products obtains through oxidative fermentation of alcohol, was prepared using different types of raw materials such as chopped vegetables and fruit peels, which otherwise would become kitchen garbage, and all of them show positive results with a very little waste as fermentation sediment that are biodegradable. While reducing the kitchen garbage by adding the culinary items, it acts as an effective post harvest management. It is suggested that post harvest losses of vegetables, cereals and fruits can be reduced using this technology.

Key words: *Saccharomyces cerevisiae*, pasteurization, flavour and aroma, mother vinegar, Storing

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INTRODUCTION

Vinegar, one of the oldest culinary products [1] discovered accidentally from spoiled wine can be prepared from a variety of fermentable substrates. It can also be prepared from alcoholic drinks such as apple wine named as "cider vinegar" [2]. It is inexpensive and requires a relatively low cost raw material. It is acetic acid and water [3], produced by oxidative fermentation of ethanol by acetic acid bacteria (*Acetobacter*), that gives the characteristic flavour and aroma to vinegar [4]. Yeasts show commensalism with bacteria. The word 'vinegar' is from the French *vin* (wine) and *aigre* (sour) which literally mean *sour wine*. Vinegar containing 4% w/v (40g/L) acetic acid with pH (2 - 3.5) is classified as a condiment [5]. Low pH inhibits the growth of food poisoning bacteria and destroys certain pathogens [6]. The strength of vinegar is quoted in grains, with 10 grains being equivalent to a concentration of 1% acetic acid [7]. Vinegar production was very much profitable in Great Britain that, in 1673 Act of Parliament, established a tax on "vinegar-beer". In the early days of the United States, the production of cider vinegar was a cornerstone of farm and domestic economy, bringing three times the price of traditional hard cider. Different types of vinegar are prepared from different raw materials and named accordingly viz. *Malt vinegar* (by malting barley), *Wine vinegar* (from red or white wine), and *Fruit vinegar* (from fruits) [4]. *Rice vinegar* (from rice), *Coconut vinegar* (from fermented coconut water), *Palm vinegar* (from the sap of the Nipa palm), *Cane vinegar* (from sugarcane juice), *Raisins vinegar* (from raisins), *Beer vinegar* (from beer), *Honey vinegar* (from honey), *Flavored vinegars* (vinegar infused with fruits), *Herb vinegars* flavoured with herbs, *Kombucha vinegar* (from kombucha, an effervescent tea based beverage), *Distilled vinegar* or *distilled spirit* or *virgin vinegar*, or *white vinegar* (distillation of any vinegar to a colourless solution of 5% to 8% acetic acid) and *Spirit vinegar* (stronger variety of 5% to 20% acetic acid, made from sugarcane or from chemically produced acetic acid). Vinegar is used in pickling, in flavouring potato chips and similar other food items, salad dressings and sauces. It is often tossed with herbs, spices, oils, sprinkled directly on vegetables and fruits for a delicious, fragrant salad. Many remedies and treatments have been ascribed to

vinegar in different cultures [8]. White vinegar gives cooling effect to sunburn; blood glucose is controlled by taking vinegar added food, or taken along with meals by reducing the glycemic index of carbohydrate food also increases satiety (the feeling of fullness) and so, reduces the amount of food consumption reduces obesity. It is used as antimicrobial, anti-infectious, as natural deodorant, hair conditioner and detangler, cervical cancer screening tool where the colour of affected tissue changes to white, as household cleaning agent because of its acidic property. Used to polish brass/ bronze, cleans grease-smearred windows and mirrors [9], as herbicide [10]. Vinegar lowers the soil's pH temporarily and breaks down quickly in water, so their residue will be gone after the first watering or rain [11]. Dilute acetic acid in the form of vinegar is harmless and has been consumed by humans for centuries. While, concentrated forms of acetic acid pose more serious health risks, such as irritation of the gastrointestinal system, respiratory system, and eyes. Esophageal injury by apple cider vinegar tablets has been reported, and because vinegar products sold for medicinal purposes are neither regulated nor standardized, they vary widely in content, pH, and other respects [12]. Long-term heavy vinegar ingestion may also cause hypokalemia, hyperreninemia, and osteoporosis [13]. Vinegar production results in very little by-products or waste and the sediments resulting from the fermentation are biodegradable and can be disposed off easily. The shelf life of a wide range of foods can be extended by storing the products submerged in vinegar by inhibition of some bacterial species [14].

MATERIAL AND METHOD

Preparation of acetic acid

Raw materials (vegetables and fruits) are first fermented to alcohol by yeast (*Saccharomyces cerevisiae*) followed by acidification with acetic acid bacteria (*Acetobacter pasteurianus/mother vinegar*), were carried out in 2011 as a post harvest management trial in BCKV, Kolkata. The vegetables (grated/chopped) and fruit peels are mixed with sugar solution (10g/L of distilled water) in 4:1 (water: pulp ratio), the pH is adjusted to 4.0 using sodium bicarbonate and ammonium phosphate (0.14g per litre). Starter culture is added at 2.7g per litre and the fermentation is allowed to take place at 25°C. Fermented extract is then filtered and acidified with acetic acid bacteria [15].

Selection of raw material

Vegetables (viz. cabbage, cauliflower, potato and carrot) and Fruits (viz. apple, watermelon and sugarcane) are used for the preparation. The selection is based on the easy availability and frequent occurrence in household kitchens, and expected diverse flavour vinegar production.

Extraction of juice

The chopped vegetables, following washing, and the core and peels of properly washed fruits are cut into small pieces, heated in a small quantity of water and crushed, pressed or squeezed [16] or grounded to get the respective juice.

pH

Acidic pH (4.0) is adjusted using sodium bicarbonate and ammonium phosphate (0.14g per litre) and with high sugar concentration the juices make them unfavourable for bacterial growth but highly suitable for yeasts and moulds. The juice may contain yeast (*Saccharomyces cerevisiae*), used in alcoholic fermentation [5].

Adjustment of sugar

Using refractometer, the sugar concentration of the vegetables and fruit juices are adjusted at 10% for optimal growth of the yeasts either by diluting with water or by adding sugar following the standard method [16].

Fermentation

It is the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substances (enzymes) of plant or animal origin" [17].

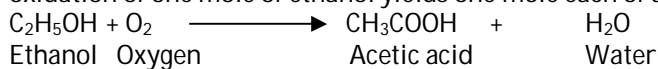
i). Alcoholic fermentation

The fruit and vegetable juice extracts were inoculated individually with *Saccharomyces cerevisiae* at 10% of the juice medium under the Laminar Flow Cabinet and incubated in B.O.D. incubator at 26±2^o C for fermentation for (5-10 days) or till the desired °Brix is reached to the lowest stabilised value.

ii). Acetic acid fermentation

Mother of vinegar (*Acetobactor aceti*) is used and the alcohol content of the fermented liquid is adjusted to (5.5-7) % by dilution with water and checking it with refractometer. It is an aerobic process, where acetic acid bacteria oxidise ethanol to acetic acid and water. Temperature is set at (26-29) °C, the ideal temperature for fermentation as the process will cease above 41^o C and below 7^o C. Low or fluctuating temperature slows the process while high temperature kills the bacteria. The acetic acid formed is checked by titrating with titrant (standard 0.5 N NaOH) from a buret to a fix volume of analyte solution

(unknown quantity of acetic acid), till the end point of colourless to pink of (acid-base) indicator, phenolphthalein is reached. Acetic acid content calculated: 1ml of 0.5 N NaOH = 0.030gm Acetic acid. The oxidation of one mole of ethanol yields one mole each of acetic acid and water.



Formation of mother vinegar (film)

After aerobic fermentation for (3 - 4) weeks, acetic acid film (called "mother") is formed which becomes heavy and falls. The vinegar is then siphoned off or strained leaving the turbid liquid (mother vinegar) to use as starter for a new batch of vinegar.

Aging of vinegar

Vinegar is allowed to age for six months to get a pleasant flavour as it gives a strong, sharp bite when it is young [18]. Upon ripening, it is kept away from oxygen to avoid further oxidation into water and carbon dioxide. Acetic acid content (5-8) % is used as table vinegar, 18% for pickling etc. examined using titration method with strong base (NaOH) and phenolphthalein as acid-base indicator.

Clarification

Through ageing, the suspended solid settles at the bottom and vinegar become clear and bright. The suspended materials are filtered to get the clear vinegar. Clarification improves the quality and appearance of the vinegar [16].

Flavouring

Different flavours are imparted using different flavouring materials (garlic, ginger etc.) by rapping in cheesecloth bag and suspending in vinegar for (1- 4) days or until the desired strength is reached.

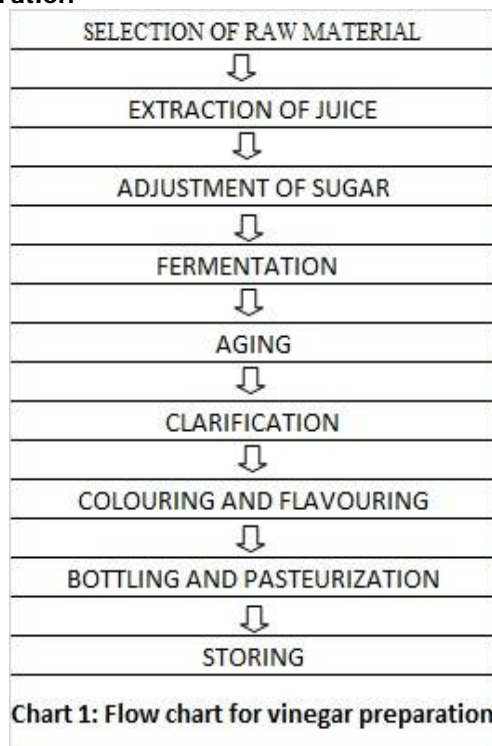
Bottling and pasteurization

Vinegar is heated before pouring it into sterilized bottles. The bottles are then placed in hot water bath for pasteurization (75-80° C for 30-40 minutes). Since bacteria can grow anaerobically, the risk of spoilage after bottling is minimised by pasteurisation after bottling [19].

Storing

After fermentation, the vinegar is stored at room temperature or in fridge indefinitely, in anaerobic conditions to prevent spoilage by the oxidation of acetic acid [20].

Flow chart for vinegar preparation



Nutritional content

Presence or absence of carbohydrate, fat, protein, and acetic acid of the vinegars are estimated following the standard methods (Table No.1).

RESULT

Vinegars from the vegetables (juice) show a very low amount of acetic acid during titration, except potato vinegar which produces a fair amount of acetic acid, while, almost all the fruit vinegars produce a fair amount of acetic acid. However, all the Vinegars show positive result in acetic acid production with variation in colour and aroma depending on the raw materials. All of them contain no protein, fat, with a very little carbohydrate (Table no. 1).

| Parameters | Method employed | Vinegars obtained from different Raw materials | | | | | | |
|----------------|--------------------------|--|---------------------|----------------|----------------|---------------|--------------------|--------------------|
| | | Cabbage vinegar | Cauliflower vinegar | Potato vinegar | Carrot vinegar | Apple vinegar | Watermelon vinegar | Sugar cane vinegar |
| Carbohydrate | Fehling's test [21] | √ | √ | √√ | √√ | √√ | √√ | √√ |
| Fat | Saponification test [22] | - | - | - | - | - | - | - |
| Protein | Biuret test [23] | - | - | - | - | - | - | - |
| Acetic acid pH | Titration method [24] | √ | √ | √√√ | √√ | √√√ | √√√ | √√√ |

Note: "√": present and low, "√√": present and medium, "√√√": present and high, "-": absent

DISCUSSION

The production of low percentage of acetic acid in vegetable vinegars (cabbage and cauliflower vinegars) may be due to their low sugar content resulting to low alcohol formation. Because, carrot and potato which comparative possess higher sugar content forms higher percentage of alcohol that converts to higher amount of acetic acid in the vinegar. All the fruits due to having higher amount of sugar form higher amount of alcohol and in turn converted to higher percentage of acetic acid in the vinegar. The study suggests that kitchen waste like fruit peels, vegetables could be employed as raw materials for vinegar preparation and could reduce waste or organic pollution in one hand while it helps in increasing culinary items without cost.

CONCLUSION

Saccharomyces cerevisiae and *Acetobacter aceti* are employed in the diluted juice extract containing at least 10% sugar, pH (4-4.5) and maintained at temperature (26-29) °C. The production of vinegar from plant juice by natural processes requires nearly (5-6) weeks. The raw materials of vinegar being readily available in the households and vinegar having a wider application ranging from culinary and flavouring, pickling and storing, medicinal and ailments, cleaning of minerals and microbes from utensils, to the field of agriculture as weedicide /herbicide and at the same time easy in making at affordable cost; its preparation at individual level can be suggested for domestic use at homes or even as a cottage industry at higher scale. It can be concluded that post harvest losses of vegetables and fruits can be converted to vinegar and assigned a commercial value.

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