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Research Article

Quality assessment of vinegar produced from pineapple wastes utilizing both laboratory-isolated *Acetobacter spp.* and reference strain *Acetobacter pasteurianus* DSM-2324

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A B S T R A C T

The research was conducted to assess the quality of vinegar made from pineapple fruit wastes using laboratory isolated Strain A (*Acetobacter spp.)* and reference strain B (*Acetobacterpasteurianus*DSM-2324). Vinegar was prepared in the laboratory. For standardization of parameters for vinegar production, different levels of carbon (10%Brix, 15% Brix, 20% Brix and 25%Brix) and different levels of pH (4.5, 5.5, 6.5 and 7.5) were applied. The highest vinegar production was found at 25% Brix and pH 5.5. The juices prepared from the wastes of pineapple were maintained at 25% Brix and pH 5.5 and two-stage fermentation of each juice was carried out first anaerobically by adding yeast strain (*Saccharomyces cereviseae*) and then aerobically by adding acetic acid bacteria (AAB) strains to the broth obtained from the first stage fermentation. In case of strain A, the total soluble solids (TSS) of the vinegar reached to 3.2%±0.00, the alcohol content became 1.0%. The pH of the vinegar dropped to 2.87%±0.02. The titratable acidity of vinegar was 5.48%±0.03. In the case of strain B, the TSS of the vinegar reached to 3.0%±0.01, the alcohol content became 1.1%. The pH of the vinegar dropped to 2.6%±0.01. The titratable acidity of vinegar was 6.01%±0.02. This study clearly indicates that pineapple fruit wastes could be used for the production of high-quality vinegar.

INTRODUCTION

Vinegar is the liquid resulting from the anaerobic fermentation process of the sugary and starchy materials by *Saccharomyces* yeast (*Saccharomyces cereviseae*) widely scattered in nature and the second fermentation process by Acetobacter bacteria which is called the "mother of vinegar" in the presence of oxygen which oxidize the alcohol resulting from the first process and produce its key ingredient acetic acid (Adames, 1998). Every year, a significant quantity of various fruits is lost as part of the excess cannot be immediately consumed by the market. Although certain alternatives to direct consumption (such as jams, fruit concentrates, fruit juices, nectars, purees, etc.) have previously been developed, plenty of fruits are left in the fields, spoils, or are picked and then disposed of as waste (Grewal *et al.*, 1988). The poor-quality fruits and their leftovers are sometimes utilized to produce vinegar. Because of its unique flavor, delightful scent, delectable taste, and nutritional and therapeutic qualities, pineapple is one of the most widely consumed and commercially significant fruits in Bangladesh. Though pineapple peels are food waste, they might be used to produce vinegar, a beneficial product that will ultimately reduce environmental pollution, preserve essential nutrients in our food, and lessen the cost of creating processed food items. Alcoholic fermentation (AF) is a fermentation step common to all vinegars. This is a biological process in which sugars, such as glucose, fructose,

and sucrose, are converted into cellular energy, ethanol, and carbon dioxide (CO_2) (Rahman *et al.*, 2024). Acetic acid bacteria (AAB) are often found in nature, and members of the Family Acetobactaceae are helpful in the commercial manufacture of vinegar (Sharafi *et al.*, 2010). These bacteria have been identified from water, dirt, vinegar, fruits, flowers, honey, sugar cane, and alcoholic beverages (Sharafi *et al.*, 2010). Bangladesh is a tropical country with a large biodiversity of fruits and microbial resources. The present study was focused on isolation and identification of AAB from various kinds of fruits as well as high acetic acid-producing strains. The native microorganisms are subjected to selective pressure in the acetification process, and those that are most suited eventually take over it. These dominant microorganisms may be good candidates to be tested for use as starter cultures. As at this point, back-slopping is a primitive precursor of the starter culture approach (Solieri and Giudici, 2009), vinegar manufacture sometimes fails due to the use of undefined starter cultures. Technological methods used for the vinegar elaboration play a vital role in the final product. One of the most used systems is the traditional method, also called the superficial, surface, or Orleans method. It is a static method that is traditionally employed for the manufacture of high-quality vinegars. The principal drawback of this method is the long period of time required to obtain a high acetic acid concentration, resulting in increased production time and costs. In order to accelerate the AAB biological process, substitute devices have been designed for the industrial production of vinegar (Tesfaye *et al.*, 2002). At the moment, the most common technology used in the vinegar industry is the submerged method (De Ory *et al.*, 1999). Alternative vinegar elaboration methods have been designed to reduce the time needed for the acetification but to replicate the quality of the final product that one obtains with traditional methods. Natural vinegar is a better food inclusion than commercial vinegar due to important amino acids produced from its fruit source and it is said to have medicinal properties for pains and stomach issues. Considering future demand, it is now time to develop a standard manufacturing technology for qualitative commercial production of vinegar from fruit wastes. Thus, the present study assessed the quality of vinegar produced from pineapple wastes utilizing laboratory-isolated *Acetobacter spp.* and reference strain *Acetobacter pasteurianus* DSM-2324.

METHODOLOGY

Selection and preparation of fruit wastes

Pineapples (average fresh weight of 1.68±0.48kg) were purchased from the local market of Bangladesh Agriculture University, Mymensingh, Bangladesh. They were kept at 22°C before undergoing the saccharification process. Following a thorough wash of the pineapples, the wastes were taken out from the edible pulp and the crown. The pineapple wastes used in the study, peel, and core, were separately processed. The peels were manually sliced using a knife into tiny pieces and then homogenized by blending them in an electric blender. Similarly, pineapple core mash was prepared.

Standardization of parameters for vinegar production

The process of producing vinegar involves fermenting fruit juices twice in succession. Following an alcoholic fermentation with a yeast strain at 30°C, an acetic fermentation using an acetic acid bacterial strain also occurs at 30°C (Seyram *et al.*, 2009). Different level of carbon in vinegar production was maintained by adjusting different sugar concentrations by adding cane sugar to fruit juices at 10°Brix, 15°Brix, 20°Brix, and 25°Brix. After adjusting the level of carbon, juices were allowed for alcoholic and acetic fermentation for vinegar production. Different level of pH in vinegar productionwas done by adjusting the fruit juice at different pH levels, which was done by adding acid (0.1N HCl) or base (0.1N NaOH) to fruit juice at pH 4.5, 5.5, and 6.5. The juices were then allowed for alcoholic and acetic fermentation for vinegar production.

Preparation of vinegar (saccharification procedure)

A mixture of 10 kg of pineapple peelings obtained by the earlier mentioned way and 5L of boiling water (100°C for 15 minutes) was used and the saccharine extracts were treated with 50ppm sulfite. Through evaporation, the resulting juice was concentrated to 20°Brix. The juice was then allowed to cool at room temperature. Organisms used for alcoholic fermentation were *Saccharomyces cereviseae* (Baker's yeast) whereas organisms used for acetic fermentation were laboratory-isolated bacteria (*Acetobacter spp.*) and *Acetobacterpasteurianus*DSM-2324 (B=Reference strain).

Preparation of yeast starter culture (alcoholic fermentation)

A small amount of must (20ml) was inoculated with viable wine yeast (*Saccharomyces cereviseae*) at a rate of 0.3 g/lit and kept for around 20 minutes to incubate in a water bath. After filtering the homogenized juice by muslin cloth manually, approximately 4.0 liters of juice was poured into a sterile plastic jerry-can and inoculated with 20 ml of the yeast starter culture. The jerry-can was tight-fitted with an airlock filled with 10 liters of distilled water (1:2.5) and kept overnight for yeast growth in the broth. This yeast culture was then inoculated to 450 ml fruit juice and incubated at 30°C for 24-hours. After that, 25 ml broths were extracted and added to 450 ml of fruit juice (20°Brix) to serve as inoculums for the alcoholic fermentation step. For 72 to 96 hours, this mixture was left to incubate at 30°C. The alcoholic fermentation was done under anaerobic conditions. The lid of the containers was tightly closed until the sugar was completely converted to alcohol. The inoculated juice was subjected to primary fermentation at ambient temperature for 7(seven) days to produce the alcohol, which was then, filtered using sterile folded muslin cheesecloth after complete primary fermentation. Fermentation was started for sedimentation and strained through cloth and clarified supernatant was taken in bottles up to ¾ capacities.

Preparation of acetic acid bacterial culture (acetic fermentation)

Fruits (pineapple, sugarcane, apple, grape, and papaya) were used for growing the acetic acid bacterial culture that was utilized for acetic fermentation. The strains were isolated and identified previously through morphological, physiological, and biochemical characteristics (Rahman *et al.*, 2024). Reference strain was collected from Deutsche Sammlung Von Microorganismen and Zellkulturen (DSMZ), Germany, and inoculated at the rate of 5% (v/v) for fermentation for

seven days in the dark under aerobic conditions. The alcohol 10.50% (v/v) was obtained after alcoholic fermentation. It was filtered and alcohol wort added for vinegar production. In order to produce vinegar, 2.8 liters of alcohol wort with a 10.50% (v/v) alcohol concentration was added to 4 liters of unpasteurized vinegar with an acidity of around 6% (w/v). For vinegar fermentation, the alcohol content of the fermented liquor was adjusted to 7-8% by diluting it with water (Byarugaba-Bazirake *et al.*, 2014). The samples were taken after every 48 hours to know the chemical compositions.

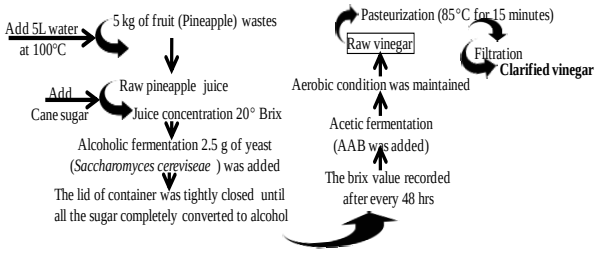

Figure 1: Schematic diagram for vinegar preparation.

Evaluation of the quality of vinegar

To assess the chemical composition of vinegar, the samples were analyzed for various parameters including TSS (°Brix), pH, specific gravity (g/ml), alcohol content (%), and titratable acidity (%) in the Food Technology Laboratory, BAU, Mymensingh, Bangladesh.

Total soluble sugars (°Brix)

The total soluble solid content of a solution is the total amount of solid dissolved in the solution, mostly sugars and soluble materials like organic and amino acids, soluble pectins, etc. This is generally an index to understand the quality of fruit juice. The amount of total soluble sugar was measured using an Abbey Refractometer. The samples were placed on the prism of the Refractometer (Atago Co. Japan) to determine total soluble sugar from the scale directly having a range of 0 to 32° Brix at room temperature.

Determination of pH

The pH of the selected samples was determined by conventional procedure with a pH meter (Ibrahim, 2002). pH meter (Hanna instruments-ORPP, *Padova, Italy*), salinity-sodium tester (China), ISO-9001 certified company; Woonsocket, RI 02895, the supplied pH 4.0 buffer solution, distilled water and 50 ml beakers.

Determination of specific gravity

To estimate the amount of alcohol created in the juice quickly and easily, the specific gravity was measured using a non-professional hydrometer. For every sample, the reading was taken three times.

Determination of ethanol

The specific gravity was measured with a non-professional hydrometer for a quick and easy estimation of the produced alcohol in the vinegar. The reading was made three times per sample. The reading was compared to a conversion table to alcohol, provided by Proulx and Nichols (2003). From the standard graph amount of ethanol in the sample was calculated.

Determination of titrable acidity

Titratable Acidity was determined using the AOAC (2005) method.

Statistical analysis

Data collected on different parameters were subjected to statistical analysis. Analysis variance (ANOVA) test was done to findout the statistical differences between different groups with the help of the wasp2 (Web Agri Stat Package) computer program.

RESULTS AND DISCUSSION

Production of acidity at different levels of carbon and pH in vinegar production

Sugar is the main source of fermentation; it must be present in adequate quantities in fruit juices for the production of quality vinegar. Different level of carbon was adjusted by adding cane sugar to fruit juices at 10°Brix, 15°Brix, 20°Brix and 25°Brix. The fermentation of juices was done by yeast strain followed by acetic acid bacterial strain in anaerobic then aerobic conditions (Figure 2a). All the Brix solutions were suitable for vinegar production but the acidity was highest (6.4%) at 25° Brix followed by 4.2% at 30° Brix, 4.1% at 20° Brix, and 3.4% at 10° Brix. It is clear that the acidity rises more in 25° Brix solution. Moryadee and Wasu (2008) reported that the variation in acetic acid production may be due to the variation in the utilization of sugar and alcohol in the fermentation medium and the acetic acid tolerance capacity of acetic acid bacteria. The result of the present study was confirmed by the findings of Divyashree (2013) who found the highest alcohol (10.2%) was observed by *Saccharomyces cereviseae* UCD522 in 25° Brix concentration among the different concentrations of sugar. The data revealed that there is a gradual increase in alcohol due to the addition of sugar to fruit juice. According to Srivastava *et al.* (1993), the isolated strains-2 produced the most ethanol at the optimal natural sugar concentration (10%) of guava pulp (5.8 (w/v), which was marginally more than the amount of ethanol produced at 20° Brix by Seyram *et al.* (2009) and by *Saccharomyces cereviseae* (5.0%) and isolates strain-1 (5.3%).

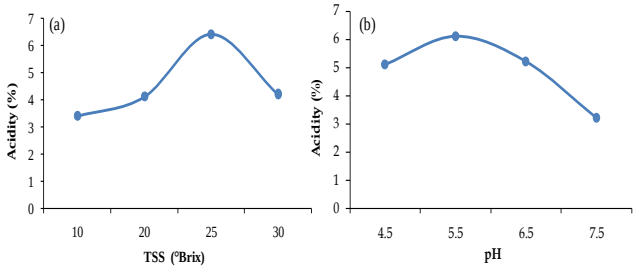

Figure 2. Production of acidity by strain A at different (a) TSS (°Brix) and (b) pH.

Different level of pH was adjusted by 0.1N HCl or 0.1N NaOH of fruit juice at pH 4.5, 5.5, 6.5, and 7.5 to determine the best pH for vinegar production (Figure 2b). After adjusting the pH (at 4.5, 5.5, 6.5, and 7.5), the fruit juices were subjected to fermentation for vinegar production. Both the yeast strain and acetic acid bacterial strain were applied during fermentation and after acidity became stable. The results showed that all the pH was suitable for vinegar production but at pH 5.5, the acidity was highest at 6.1% followed by acidity 5.2% at pH 6.5, the acidity 4.9% at pH 4.5 and the acidity was 3.2% at pH 7.5 (Figure 2b). The results indicate that the starting pH 5.5 is most suitable for vinegar production. After knowing the standard value of TSS and pH for fermentation, vinegar was produced from the wastes of pineapple. Divyashree (2013) found that the higher alcohol (10.0) was obtained at pH 7.5 by *Saccharomyces cereviseae* and was reduced to pH 3.23. Olasaupo and Obayori (2003) found that pH gradually decreased from an initial pH of 7.3 to 3.5 at the end of the fermentation. The pH of vinegar depends upon the acids and sugar content of vinegar. Since vinegars were prepared from supplementation of organic acid and sugar content, differences in pH were noticed. Seyram *et al.* (2009) reported that increased acetic acid production may be due to the decrease in the pH of vinegar.

Quality of vinegar (alcoholic fermentation)

The juices prepared from pineapple wastes were maintained at 25°Brix and pH 5.5. The fermentation of juice (300ml) was carried out anaerobically by adding yeast strain (*Saccharomyces cereviseae*) which was previously inoculated and incubated for 24 hours at 30°C. The fermentation of juices obtained from pineapple wastes (25°Brix) by *Saccharomyces cereviseae*strain presented complete sugar utilization in 96 hours with the highest ethanol production of 10.50% (v/v) and the TSS reduced to 7.4 (°Brix). A clear relationship between the formation of ethanol and a decline in pH, with a pH value from 5.5 to 3.6, was also demonstrated by the data (Figure 3). The cause of these pH decreases was thought to be the fermentation's emission of CO_2 and organic acids (Dombek and Ingram 1987; Fu and Peiris 2008; Uppal 2009; Sankhla *et al.*, 2012). Muhammad *et al.* (2000) have also previously reported on Such types of retention.

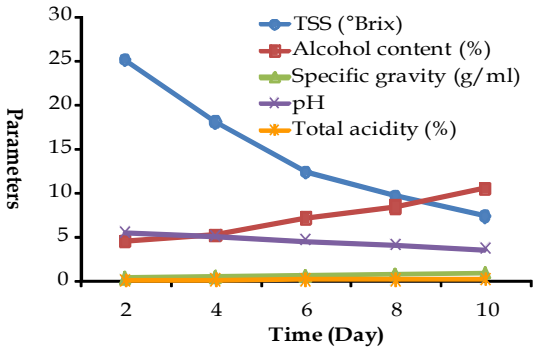

Figure 3. Parameters after10 days of alcoholic fermentation

The result of this study corresponds with that of IIha *et al.* (2000), who found that the alcohol level of the five replicates in their experiment using bee (*Apismellifera*) honey to produce vinegar ranged from 7.97% to 8.15% (v/v).The alcoholic content of 7.0–10.0% (v/v) was noted by Vidal (1983) when he prepared mead using sugarcane honey and these data are also comparable. Bhatt *et al.* (1987) found similar results in alcohol production when they used guava juice and banana to produce ethanol with *Saccharomyces cereviseae* as inoculums.

Quality of vinegar (secondary fermentation)

Two sources of acetic acid bacteria strains A and B, were used to perform secondary fermentation on all the alcohol that was released by the settling yeast. When the ethanol was converted to acetic acid during acidification, the acidity rose, but no appreciable variations were seen across the various bacterial cultures. It is worth noting that it was difficult to achieve a homogeneous airflow in all bottles. These factors resulted in a large variation of the acetification in each of the three replications within each treatment. Both strain A and strain B were inoculated and pre-cultured into smaller volumes before the fermentation was performed. The decrease in alcohol concentration corresponded to the gradual rise in acetic acid concentration which accumulated from 10.50 to 1.0% (v/v) for strain A over a fermentation progress period of 21 days. The pH of the vinegar during the secondary fermentation was recorded to decrease slightly from pH 3.6 to 2.8 and the acidity became 5.48% when the specific gravity was 1.02 gm/ml (Table 1 and Figure 4a).

Table 1. Physiochemical properties of alcohol and vinegar (For strain A and Strain B)

Parameters	Alcohol			Vinegar		
	Strain A	Strain B		Strain A	Strain B	
TSS (°Brix)	7.4±0.15	7.4±0.15	NS	3.2±0.00	3.00±0.01	NS
Alchol content (%)	10.50	10.50	NS	1.00	1.10	NS
Specific gravity (g/ml)	0.94±0.23	0.94±0.23	NS	1.02±0.01	1.032±0.02	NS
pH	3.60±0.14	3.60±0.14	NS	2.80±0.02	2.60±0.01	NS
Total acidity (%)	0.18±0.01	0.18±0.01	NS	5.48±0.03	6.01±0.02	Sig

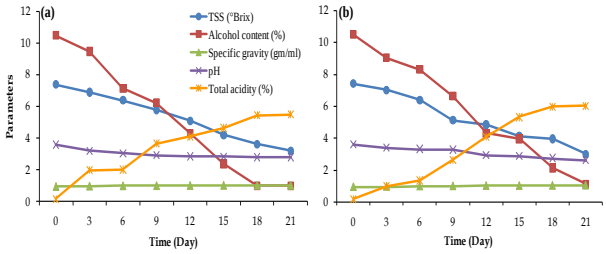

Figure 4. Parameters after 21 days of acetic fermentation by (a) strain A and (b) strain B.

The decrease in alcohol concentration corresponded to the gradual rise in acetic acid concentration which accumulated from 10.50 to 1.1% (v/v) for strain B over a fermentation progress period of 21 days. The pH of the vinegar during the secondary fermentation was recorded to decrease slightly from pH 3.6 to 2.6 and the specific gravity reached1.032 g/ml. The titratable acidity of vinegar after 21 days of fermentation became 6.01(%) in the case of strain B (Table 1 and Figure 4b).

In the case of strain A, the pH of the vinegar obtained from pineapple wastes was 2.80±0.02. In the case of strain B, the pH of the vinegar obtained from pineapple wastes was 2.60±0.01. Results obtained for pH were quite similar to the findings of Qais and Abass (2016) who reported that the pH of the vinegar samples namely S_1, S_2, S_3, and S_4 were 2.93, 3.13, 3.71, and 3.43, respectively. Results were close to the findings of Smano *et al.* (1991) for samples of vinegar which were produced by barley malt as it ranged between 3.36-3.75. Results were identical to what has been obtained by Chang *et al.* (2005) for samples of grape vinegar as the value was 3.36.

In the case of strain A, the TSS (°Brix) of vinegar obtained from pineapple wastes was 3.21±0.00. In the case of strain B, the TSS (°Brix) of vinegar from pineapple wastes was 3.00±0.01. Qais and Abass performed a study of some chemical and physical characteristics of vinegar produced by the malt of some varieties of maize, zehdi dates, and grapes and found that the TSS (°Brix) of five samples namely S_1, S_2, S_3, S_4, and S_5 were 3.8, 3.7, 3.5, 2.9 and 4.3 respectively. Comparing the proximate values, mineral elements, and heavy metal contents of three local fruit vinegar with apple cider vinegar, Faznira, and Seri (2014) found that the TSS (°Brix) for Kelubi vinegar, Rambutan vinegar, Dokong vinegar, and apple cider vinegar are, respectively, 12.40±0.00, 22.00±0.00, 20.20±0.00, and 3.60±0.00. Seyram *et al.* (2009) found the TSS is 5.3°Brix in a study of pineapple peelings into vinegar by biotechnology, which is in comparison with the results of the present study.

In the case of strain A, the titratable acidity (%) of vinegar from pineapple wastes was 5.48±0.03. In the case of strain B, the titrable acidity of vinegar from pineapple wastes was 6.01±0.02. Faznira and Seri (2014) reported that the titrableacidity (%) of Kelubi vinegar, Rambutan vinegar, Dokong vinegar, and Apple cider vinegar are 5.33±0.07, 1.78±0.01, 1.74±0.02 and 6.34±0.35 respectively. Oscar *et al.* (1975) found the acidity of vinegar from pineapple are 4.22, 5.6, and 3.5. Umaru *et al.* (2015) found in the production of vinegar from pineapple peel wine using *Acetobacter* species that the acidity was 3%. The variations are similar to one another, but since acetic acid bacteria are known to be highly sensitive to air interruption during the oxidative process, this might be explained by a stoppage in the air supply during the process. Ilha *et al.* (2000) found acetic acid (%w/v) is 2.322±0.227. Yash *et al.* (2017) found the titratable acidity 4.37, 3.32, and 6.38 for sugarcane, coconut, and pomegranate respectively inthe manufacturing of cost-effective vinegar from different fruit products.

CONCLUSION

Preparation of vinegar from pineapple wastes both with laboratory isolated strain and reference strain DSM 2324 and their quality assessment were done by standardization of parameters for vinegar production. On the basis of the experiments, wastes of pineapple were used for the standardization of parameters for vinegar production. The acidity rises more in 25° Brix solution and the starting pH of 5.5 is most suitable for vinegar production. The fermentation of the juices (25°Brix) by *Saccharomyces cereviseae* strain presented the sugar utilization with the highest ethanol production of 10.50% (v/v). In the case of strain A, the pH of vinegar reached2.80±0.02. In the case of strain B, the pH of vinegar became 2.60±0.01. In the case of strain A, the TSS (°Brix) of vinegar is 3.2±0.00. In the case of strain B, the TSS (°Brix) of vinegar was 3.00±0.01.In the case of strain A, the titrable acidity of vinegar was 5.48±0.03. In the case of strain B, the titratable acidity of vinegar was 6.01±0.02. Therefore, in such a way, vinegar can be produced by utilizing fruit wastes in Bangladesh.

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Author's contribution

Conceptualization: Rahman M; Methodology: Rahman M, Uddin MB, Aziz MG; Experiment management and data collection: Rahman M and Siddiki MSR; Data analysis: Rahman M and Siddiki MSR; Writing-original draft preparation: Rahman M, Haque MR; Writing-review and editing: Siddiki MSR; Supervision: Uddin MB, Aziz MG. All authors have read and approved the final manuscript.

Data availability

Data are contained within the article.

Conflict of interest

None to declare

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