Production of Vinegar from Pear Juice and Comparative Analysis of its Quality with Apple Juice Vinegar

ABSTRACT

Vinegars are the product of scalar fermentations carried out by several groups of microorganisms acting at different moments in time. Apple vinegar is made with apple juice or concentrated Apple juice (CAJ) through a double fermentation: alcoholic and acetic. Vinegar was successfully produced from the juice extracted from pear using yeast and acetic acid bacteria. This study presents a summary of research concerning the quality of vinegar made from pear and apples by using double distillation method. Juice is extracted from both the apple and pear and was analysed for several parameters like pH, brix, specific gravity, Total Reducing Sugars (TRS), Reducing Sugars (RS), titratable acidity, alcohol percentage and Total Dissolved Solids (TDS). pH of pear and apple juice was found to be 4.82 and 4.35 respectively. pear and apple juice had the Brix of 8.5° and 12° respectively. The TRS of pear and apple juice was found to be 10.2%and 11.87 % respectively. Quality characteristics between pear vinegar and apple vinegar were investigated which includes pH, titratable acidity and alcohol percentage. The alcohol percentage of pear vinegar is slightly less than apple vinegar but the titratable acidity of pear vinegar was slightly more as compared to apple vinegar. Also pH of pear vinegar and apple vinegar are found to be 3.60 was 3.58 respectively. Acetic acid bacteria were isolated from Pear and apple vinegar prepared, using Frateur medium and Glucose Yeast Peptone (GYP) agar medium. Aceto Acetic Bacteria (AAB) isolated was confirmed to be Gram negative rods through Grams staining. The study reveals that pears being a seasonal fruit and cheaper compared to apples can be used to prepare healthy vinegar of best quality by a simple double fermentation method.

Keywords: Double fermentation; pear vinegar; apple vinegar; acetic acid bacteria and grams staining.

1. 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 [1]. "Vinegars are commonly used for pickling of fruits and vegetables and in the preparation of mayonnaise, salad dressings, mustard and other food condiments. Although useful as a food ingredient for flavor and functional properties, the potential health benefits of vinegar varieties are leading researchers to further consider this long used food product" [2,3].

"As defined by FAO/WHO, vinegar is a liquid, fit for human consumption, produced exclusively from suitable products containing starch and/or sugars by the process of double fermentation; first alcoholic and then acetous. Thus, depending on the raw material used, it can be of different types. White vinegar is most ordinarily found in Indian kitchens. This vinegar is produced using grain-based ethanol or acidic corrosive, which is blended with water to make it more solvent and appropriate for cooking" [4]. "Rice vinegar a light yellow assortment is better in taste when contrasted with different vinegars. It is produced using fermented rice and is generally used to marinate meats or make plunges and dressings". Yano *et al.,* [5]. "Balsamic vinegar is made directly from grapes and contains no liquor. It is blackish in shading and the best of the apparent multitude of assortments of vinegar. It can be utilized for a plate of mixed greens dressing or used as a sauce/plunge for finger nourishments" [6]. "Apple Cider vinegar is produced using apple juice and accordingly, contains an unmistakable fruity tone. It may be utilized very well to cook non-vegan dishes, oats and smoothes. Other than adding mellow acidity to dishes, apple juice vinegar gives different other medical advantages, particularly for skin and hair" [7]. "Red wine vinegar is a more impactful and acidic than white wine vinegar. Wine vinegars are generally used to get ready European dishes like sauted food

vegetables and Mexican Salsa" [8]. "Traditional vinegar is made from cereals and has been consumed for a long time. Another type of vinegar, fruit vinegar, made from fruit or fruit juices, has become increasingly popular in recent years because consumers are paying more attention to the functional properties of food products" [9]. "Fruit vinegars are popular all over the world due to their good flavor and health benefits. Fruit vinegars can easily be made from the peels and cores of apples and pears. Apple vinegar is part of vinegar fruit apples obtained by biotechnological process of double fermentation, alcoholic and acetic. Prior to the start of the acetic fermentation apple wine is subjected to alcoholic fermentation which is carried out with yeast, thus obtaining the amount of necessary alcohol to produce acetic acid" [10]. "Unpasteurized or organic Apple cider vinegar (ACV) contains mother of vinegar is fermented juice from crushed apples. Like apple juice, it likely contains some pectin, vitamin B1, vitamin B2, vitamin B6, Biotin, Folic acid, Niacin, Pantothenic acid and vitamin C" [11].

"The acetic acid bacteria (AAB) are mesophilic obligate aerobes that oxidize sugars, sugar alcohols and ethanol, with the production of acetic acid as the major end product. During acetic acid production, ethanol is almost quantitatively oxidized to acetic acid. The AAB exhibit resistance to high acetic acid concentrations and low pH" [12]. "Vinegar was known by ancient civilisations and used in folk medicine in wound treatment, as well as a hand-washing agent, to prevent infection. Nowadays, it is commonly used in the preparation of pickles, salad dressings and other food products. Vinegar has also become recognised for its functional properties, such as antibacterial activity, blood pressure reduction, antioxidant activity, reduction in the effects of diabetes and prevention of cardiovascular disease" [13]. "Vinegar was proved to reduce body weight, BMI and body fat mass in obese subjects" [14]. A study indicates that apple cider vinegar consumption along with restricted calorie diet can decrease appetite, hip circumference, VAI, plasma triglyceride, total cholesterol concentration and also increase HDL-C level in overweight or obese subjects [15]. With regular and continued use, this wonderful liquid helps restore and balance the body's pH, taking it from acidic to neutral in a short span of time [11] A pear is a mild, sweet fruit with a fibrous centre. *Pyrus communis L* is the scientific name of pear [16]. They are rich in essential antioxidants, plant compounds and dietary fiber. In India, Pear is cultivated in Himachal Pradesh, Jammu and Kashmir and U.P [17]. Pear is one of the most popular fruits in the world due to its pleasant taste and nutritional value [18]. It is also one of the crops with the oldest known cultivation history [19]. "China is the largest producer of pear, contributing more than half of the world production with over 15 million metric tons a year" [20]. "Pear is a nutrient dense food, providing a significant amount of carbohydrates, vitamins, minerals and phenolic compounds. Due to the high amount of dietary fibre and low in acidity, pears are very popular in the production of baby food purees and multi fruit nectars containing high acidic fruits" [21]. The preliminary study revealed that properties of pear juice are found to be similar to that of apple juice. Hence the study is further planned to produce vinegar from pear juice and apple juice and compared the quality of both.

2. MATERIALS AND METHODS

The present study was carried out at Biochemistry Division, National Sugar Institute, Kanpur. Chemicals used were of high-grade reagents.

2.1 Production of Pear Juice Vinegar

2.1.1 Collection of pear

Pears used in this study were obtained from local markets in Kalyanpur, Kanpur, Uttar Pradesh. The pears were selected according to the required degree of ripeness needed at the time of purchase.

2.1.2 Preparation of pear juice

There are three main ways to make pear juice - with a juicer, blender or manually (machine free). For preparation of pear vinegar, pear juice is squeezed manually from pear. One kg of pear was taken and washed thoroughly. Then, using a grater, pulp was taken out by grating the pear. With the help of muslin cloth, juice was squeezed out from the pulp. Some amount of distilled water was also added in the pulp to get more juice from the remaining pulp. The pear juice obtained was measured and collected in a container; properly sealed and stored at room temperature for further analysis.

2.1.3 Analysis of pear juice

The pear juice obtained was analysed for the following pH, Brix, specific Gravity, total reducing

sugars, reducing sugars, alcohol Percentage, titratable acidity and total dissolved solids.

2.1.4 Preparation of vinegar from pear juice

Pear juice was sterilized to prevent the growth of unnecessary microorganisms. Following sterilization, pear juice was cooled and added with active dry yeast (Saccharomyces cerevisiae) of 2g/100 mL pear juice. Yeast was first added to the sterile luke (this is right) warm water and was kept for 10 minutes and then transferred to conical flask containing pear juice. The pear juice containing yeast was kept on magnetic stirrer for shaking for two hours at 28 ° C. After shaking, it was kept anaerobically in a dark place for 24 – 72 hours. Distillation was done to check alcohol percentage of the fermentation broth after 48 hours. The particles suspended in fermentation broth were separated by centrifugation. 10 mL of white vinegar was added to the 100 mL fermented pear juice to start acetic acid fermentation.

Interval shaking was carried using magnetic stirrer at 200 rpm for 8 hours per day for 3 consecutive days. The pH, alcohol percentage and titratable acidity parameters were analysed at 24 hrs,48 hrs and 72 hrs. Pear Vinegar thus produced was stored in the refrigerator.

2.1.5 Production and analysis of apple vinegar

The method used for production and analysis of pear vinegar was also used for the production and analysis of apple vinegar using apples procured from local market.

2.1.6 Isolation of acetic acid bacteria from vinegar

For initial isolation of AAB from pear and apple vinegar prepared, Frateur medium was used for spread plate technique. The bacterial growth observed after 48 hrs was isolated using Glucose Yeast Peptone (GYP) agar medium with repeated slant preparation. Isolated AAB was finally subjected to Grams staining. The composition of Frateur medium and GYP agar medium is given in Table 1 and 2.

Table 1. Composition of frateur

Yeast extract	1%
Agar.	2%
CaCO3.	2%
Distilled water	100ml

Table 2. Composition of GYP agar medium

Peptone.	10 gm
Yeast extract.	5gm
Dextrose.	20 gm
Agar.	15 gm
Distilled water.	1000

3. RESULTS AND DISCUSSION

Pears and apples of fine quality were purchased from a local market of Kalyanpur Kanpur. Both fruits were washed with tap water, then dried and cut into pieces. Stem, seed and bruised portions were discarded. Juice was extracted with the help of grater. The photos of grated pear, extraction and extracted pear juice are given in the Fig. 1.

The Fig. 2 represents the grated apple, extraction and extracted apple juice. The pH, temperature, specific gravity, brix, total reducing sugars, reducing sugars, titratable acidity, alcohol percentage and the total dissolved solids in apple juice and pear juice are reported in Table 3.

Most apple juices have a pH between 3.0 for the more acidic juices and up to about 4.5 for the juices that contain very little acidity [22]. Apple juice made from flawless raw materials is generally alcohol-free. The alcohol content in semi-finished apple juice is 0.2...0.4 % [23]. The titratable acidity of apple juices in terms of malic acid, as a rule, is 5-9 g/1, but it can be from 3.5-14.0 g/1 and higher [24]. The obtained results show the similarity in most of the parameters between both the juices – pear and apple juice. Vinegar from pear juice and apple juice were made and the results of quality parameters analysed are presented in the Table 4.

Fig. 1 (a). Grated pear b) extraction c) pear juice

Fig. 2 (a). Grated apple b) extraction c) apple juice

Table 3. Analysis of pear and apple juice

S.NO	PARAMETERS	PEAR JUICE	APPLE JUICE
1.	pH	4.82	4.35
2.	Temperature	29.9°C	30.0°C
3.	Specific gravity	1.028	1.036
4.	Brix(°)	8.5	12.0
5.	Total reducing sugars (%)	10.2	11.87
6.	Reducing sugars (%)	6.8	8.14
7.	Titrable acidity in g/l (w/v Malic acid)	4.4	3.9
8.	Alcohol %	Nil	Nil
9.	Total dissolved solids	1.03 ppm	1.04ppm

Table 4. Analysis of vinegar fermentation broth before acetic fermentation

S.No	Parameters	Pear Vinegar	Apple Vinegar
1.	pH	4.20	4.17
2.	Temperature	24°C	24°C
3.	Titratable Acidity in g/l (w/v Malic acid)	2.3	2.01
4.	Alcohol%	5.40%	5.29%

Total titratable acidity and alcohol percentage were found to be increased during ethanol fermentation (Table 3 Vs Table 4). Budak *et al.,* [25]. revealed that the total sugar content decreased with increase in alcohol fermentation by *Saccharomyces cerevisiae* during the apple cider vinegar preparation.

The pH variations during acetic acid fermentation is recorded in Table 5.

The final pH of pear vinegar and apple vinegar are found to be 3.60 was 3.58 respectively. The ACV can be delivered by a two-venture aging cycle, and this cycle is described by the presence of acidic corrosive at a fixation

equivalent to or above 4%. Juice vinegars are commonly 5-6% acidic corrosive. The pH of vinegar will rely upon acidic corrosive fixation and is commonly between 2 – 3.5 [26]. The alcohol % was found to be increased during the acetic acid fermentation as given in the Table 6.

Jeong *et al*., [27] Seo *et al.*, [28] reported that the alcohol content differs a lot between different countries: For example, in Ireland the alcohol content can reach 9-10% v/v, while in North America the cider has 5 - 6.5% alcohol and the length of fermentation is close to one week. Titrable acidity variations with time in pear and apple vinegar are given in Table 7.

Table 6 and Table 7 shows alcohol% and titratable acidity of both the vinegars. The alcohol% of pear vinegar is less than apple vinegar upto 48 hours but the titratable acidity of pear vinegar was more as compared to apple vinegar. Fig. 3 depicts the fermentation process with interval shaking of broth using magnetic stirrer.

Table 5. pH of vinegar fermentation broth during acetic fermentation

S.NO.	Duration	Pear Vinegar	Apple Vinegar
1.	24 hours	3.63	3.61
2.	48 hours	3.60	3.60
3.	72 hours	3.60	3.58

Table 6. Alcohol percentage of Fermentation broth during acetic acid fermentation

S.No	Duration	Alcohol % in pear juice vinegar	Alcohol % apple juice vinegar
1.	24 hours	3.39%	4.33%
2.	48 hours	3.53%	4.09%
3.	72 hours	4.54%	4.04%

Table 7. Titratable acidity of fermentation broth during acetic acid fermentation

S.NO.	Duration	Pear Vinegar	Apple Vinegar
1.	24 hours	8.41	7.81
2.	48 hours	8.45	7.75
3.	72 hours	8.45	8.1

Fig. 3. Interval shaking on magnetic stirrer

Adriana Dabija [10] reported the total acidity (% acetic acid) range as 3.9 -9 in the Study concerning the quality of apple vinegar obtained through classical method. Hence, the results of this study is consistent with the earlier studies.

The pear and apple vinegar produced are shown in the Fig. 4. a and Fig. 4. b. Pear vinegar is extremely light and a little bit sweet in taste. The present preliminary studies reveal that pear vinegar prepared is similar to apple cider vinegar, hence it will have the same beneficial properties.

Isolation of Acetic acid bacteria (AAB) is done on Frateur medium by using spread plate technique.

The bacterial colonies are formed on petri dishes as shown in Fig. 5.

The bacterial colonies of Acetic acid bacteria (AAB) formed on petri dishes are used to prepared GYP agar slants in order to get the pure culture of AAB. Fig. 6.a and 6. b shows the pure culture of AAB grown on agar slants from apple vinegar and pear Vinegar respectively.

The pure culture of AAB obtained is subjected to Gram Staining experiment. The gram negative rods in chains are observed in microscope and the image is given in Fig. 7.

The bacteria appear pink in colour and hence the isolated AAB are gram negative bacteria. Gram-negative bacteria are bacteria that do not retain the crystal violet stain due to high lipid content of the cell wall. The shape of the bacteria appeared as rods.

AAB (Acetic acid bacteria) is a gathering of microorganisms in the family Acetobacteriaceae. AAB are obligated aerobes that stain as Gram-negative or Gram variable, are catalase-positive, and oxidase negative. The non-spore-forming cells are the bar to ellipsoidal-formed [29].

Fig. 4 (a). Pear vinegar b) Apple vinegar

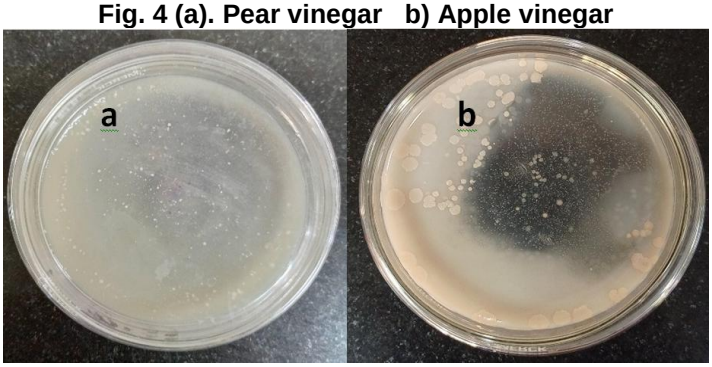

Fig. 5 (a). AAB from pear vinegar b) AAB from apple vinegar

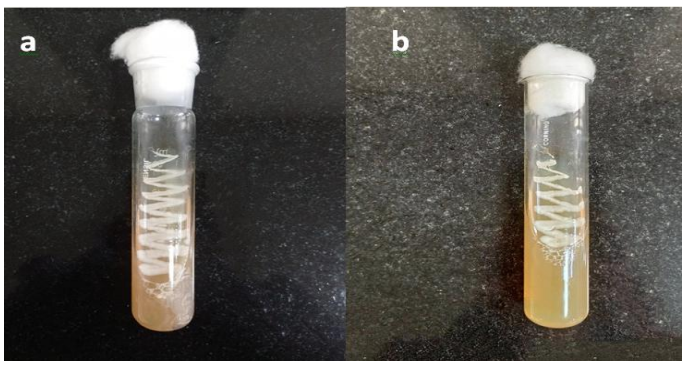

Fig. 6 (a). AAB of pear vinegar on GYP slants b) AAB of apple vinegar on GYP slants

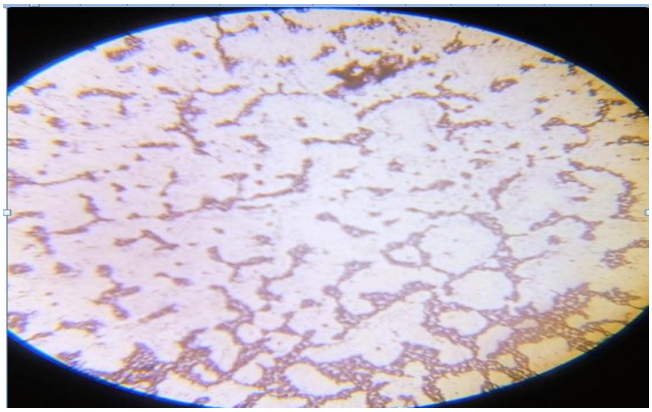

Fig. 7. Gram negative rods of isolated AAB

4. CONCLUSION

Vinegar is an acidic liquid produced through the fermentation of ethanol by acetic acid bacteria. It is used in cooking not only for its flavour qualities but also for its chemical properties. It can be made from a variety of base ingredients that contribute their own unique characteristics to the vinegar and add extra flavour and vibrancy to foods they are used with. Apple vinegar made from fermented apple juice is one of the most popular vinegar. It's used as an ingredient in foods like salad dressings, pickles and marinades. Since apple and pear belongs to the same Rosaceae family. preparation of vinegar from pear was explored with success.

From the study it was found that good vinegar can be prepared from pears and the quality is found to be same as that of vinegar made from apple. Hence the benefit which apple vinegar claims might also be present in pear vinegar like weight loss, controlling type 2 diabetes, blood sugar and cholesterol. It can also prevent growth of harmful bacteria on foods.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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