Original Research Article

PRODUCTION OF VINEGAR FROM PEAR JUICE &COMPARATIVE ANALYSIS OF ITS QUALITY WITH INDIGENOUS 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 vinegaris 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, TRS, RS, titratable acidity, alcohol percentage and 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. 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 GYP agar medium. AAB isolated was confirmed to be Gram negative rods through Grams staining.

Key Words: 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 **(Cruess 1958).**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 **(Turker 1963; Tan 2005).**

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 **(Pizarro *et al.,* 2008).** 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.,* 1997).** 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 **(Gullo *et al.*,2009).** 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 **(Zou *et al.,* 2012).** 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 **(Lee *et al.,* 2013).** 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 **(*Qing et al.,* 2019).** 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 **(Adriana Dabija *et al*.,2014).** 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 **(Singh Akanksha *et al.,* 2017).**

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.AAB exhibit resistance to high acetic acid concentrations and low pH **(Raspor *et al.,* 2008).** Cider vinegar and honey are used for the treatment of arthritis and also ACV is used externally for painful joints. Apple cider vinegar plays an important role in controlling the sugar levels in the blood. Moreover, it helps to lose weight, which is important, considering obesity drivers of diabetes. Simply drink a glass of water containing two teaspoon ACV before each meal. This concoction also known to relieve stomach ache. When it is used medicinally it helps the body to rid itself from harmful toxins. It has wonderful disinfecting properties as a natural bad germ fighter, and is a biodegradable substance that does not pollute the environment. 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 **(Singh Akanksha *et al*.,2017).** A pear is a mild, sweet fruit with a fibrous center. Pyrus is the scientific name. 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.Pear is one of the most popular fruits in the world due to its pleasant taste and nutritional value **(Xie *et al.,* 2007)**. It is also one of the

crops with the oldest known cultivation history **(Wu *et al.*, 2013)**. China is the largest producer of pear, contributing more than half of the world production with over 15 million metric tons a year **(FAO, 2012)**. 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 **(Markowski *et al.*, 2012).** The properties of pear juice are found to be similar to that of apple juice. Hence the study is planned toproduce vinegar from Pear juice and apple juice and compared the quality of both.

2. MATERIALS & METHODS

The present study was carried out at Biochemistry Division, **National Sugar Institute, Kanpur.** Chemicals which have been used in study of sweet sorghum spent wash were of high-grade reagents.

2.1Production 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 a 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. Took 1 kg of pear and were washed so that there was no dust on them.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.

2.1.3Analysis 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, TDS.

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/100ml pear juice. Yeast was first added to the sterile luke 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 are separated by centrifugation and

collected the supernatant. 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. pH, alcohol percentage and titratable acidity parameters are analysed at 24 hrs,48 hrs and 72 hrs. PearVinegar thus produced was stored in the refrigerator.

2.1.5 Isolation of Acetic Acid Bacteria from Indigenous 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.

Table 1 Composition of Frateur medium

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 ml

3. RESULT 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.

Figure 1: a) Grated Pear b) Extraction c) Pear juice

Figure 2: a) Grated Apple b) Extraction c) Apple juice

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

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

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 **(Musshoff*etal.*, 2010)**Apple juice made from flawless raw materials is generally alcohol-free. The alcohol content in semi-finished apple juice is 0.2...0.4 % **(Jolicoeur,2011).**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 **(Finglas*etal.*,2015)**The obtained results show the similarity in most of the parameters between both the juices – Pear and Apple Juice. Hence, an attempt is made to prepare Vinegar from pear juice and the quality of vinegar produced from apple and pear is studied.

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 is found to be increased during ethanol fermentation. **Budak *et al.*, 2010**, in his study, revealed that the total sugar content decreased with increase in alcohol fermentation by *Saccharomyces cerevisiae* during the apple cider vinegar preparation.

Table5: 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

The final pH of pear vinegar and apple vinegar are found to be 3.60 was 3.58 respectively. 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 **(Webb, 1983).**

Table 6: Alcohol percentage of Fermentation broth during acetic fermentation

S.NO	DURATION	PEAR VINEGAR	APPLE VINEGAR
1.	24 hours	3.39%	4.33%
2.	48 hours	3.53%	4.09%
3.	72 hours	4.54%	4.04%

Jeong *et al.*, 1999; Seo*et al.*, 2001 reported that the alcohol content differs a lot between different countries: i.e. 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.

Table 7: Titratable acidity of Fermentation broth during acetic 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

Table 4 and Table 5 shows alcohol% and titratable acidity of both the vinegars. The alcohol% of pear vinegar is less than apple vinegar but the titratable Acidity of pear vinegar was more as compared to apple vinegar.

Figure 3 Interval shaking on magnetic stirrer

Adriana Dabija (2014) 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.

Figure 4 a) Pear vinegar b) Apple vinegar

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. Pear vinegar not to dissimilar from apple cider vinegar, it has 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.

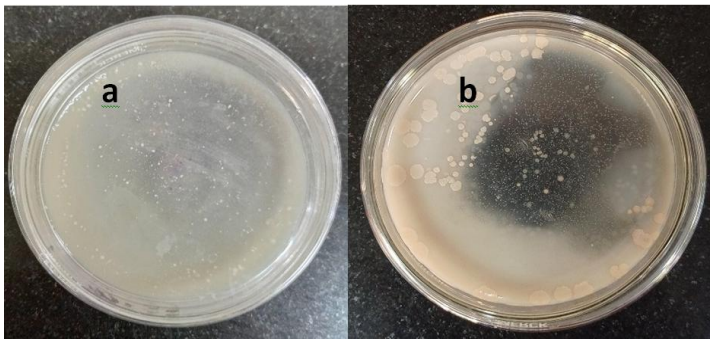

Figure 5 a) AAB from pear vinegar b) AAB from apple vinegar

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. Figure 6.a and 6. b shows the pure culture of AAB grown on agar slants from apple vinegar and pear Vinegar respectively.

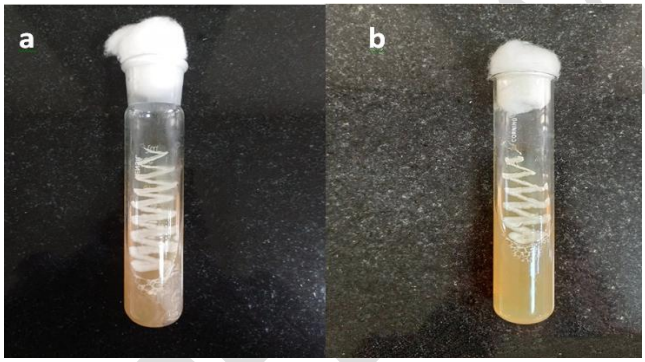

Figure 6 a) AAB of pear vinegar on GYP slants b) AAB of apple vinegar on GYP slants

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

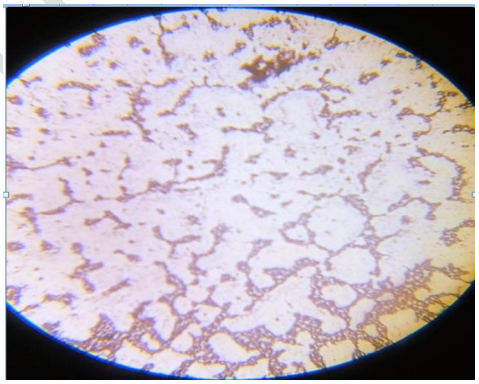

Figure 7 Gram negative rods of isolated AAB

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 **(Sengun *et al.*,2011).**

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 is attempted and succeeded.

Prior to preparation of vinegar, Juice is extracted from both the apple and pear and was analysed for several parameters like pH, brix, specific gravity, TRS, RS, titratable acidity, alcohol percentage and 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 12respectively. TRS of Pear and apple juice was found to be 10.2 and11.87 % respectively.

Vinegar from both the fruits is prepared at the same time following the same procedure. The preparation of vinegar from both apple and pear includes two step fermentation processes. The first stage is alcoholic fermentation (AF), which converts sugar into ethanol by yeast (usually Saccharomyces). The second step is acetic acid fermentation (AAF), in which ethanol are oxidized to acetic acid by acetic acid bacteria.

Both apple and pear vinegar prepared had a sweet aroma and had the pH of 3.58 and 3.60 respectively. The two main parameters in vinegar analysis is alcohol percentage and titratable acidity. The alcohol percentage of pear vinegar was found to be 4.54and that of apple vinegar was found to be 4.04%. The titratable acidity of vinegar was measured in terms of acetic acid. Titratable acidity of pear vinegar was found to be 8.45% which is slightly more compared with that of apple vinegar that have 8.1%. 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 be also 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.

From the vinegar prepared from pear and apple, Acetic acid bacteria were isolated using Frateur medium and GYP agar medium. AAB slants are stored for future for further studies. AAB isolated was confirmed to be Gram negative rods through Grams staining.

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