ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Production and Evaluation of Wood Apple Vinegar

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ABSTRACT

Vinegar is a product of double fermentation including the primary stage by anaerobic conversion of fermentable sugars to ethanol by Saccharomyces species, and the second one is the oxidation of ethanol to acetic acid by means of micro-organisms, commonly used by *Acetobacter aceti*. The *Feronia limonia*variety of wood appleswas cut with a knife, crushed with a mixer to make them juicy, sampled, and immediately frozen followed by pre-treatment and depectinization). The excellent result was acquired at 0.0004 % (w/v) of wood apple. At 48 hrs., 10% yeast cellular concentration produced 7.6% of alcohol. Alcohol manufacturing steadily reduced sugar concentration from around13% to 11%.After fermentation, the vinegar produced a colorof pale brownish. The results confirmed that acetic acid concentrations extended with an increase in the inoculum degree tested. The best acetic acid concentrations of 5.32 % and 5.11 % were discovered on the five and 10 % inoculum stages, respectively for the very best alcohol-contained pattern (SET C). The numerous parameters assessed evaluate favorably with the usual magnitudes.

Keywords: Acetobacter aceti, Fermentation, Saccharomycescervisiae, Wood apple, Vinegar

INTRODUCTION

Vinegar was known internationally as a food-preserving flavored molecule[1]. Vinegar is taken into consideration as “a liquid match for human intake, comprised of a suitable raw material

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of agricultural beginning, containing starch, sugars, or starch and sugars by the method of double fermentation, alcoholic and acetous, and consists of a certain quantity of CH_3COOH"[2]. Vinegar, a conventional acidic condiment, is widely made from carbohydrate-rich diets like rice, malt, banana, apples, wine, and other agricultural molecules[3]. Vinegar is a product of double fermentation. Vinegar fermentation in the primary stage is by anaerobic conversion of fermentable sugars to ethanol by yeasts, usually Saccharomyces species, and the second one is the oxidation of ethanol to acetic acid by means of micro-organisms, commonly used Acetobacter aceti. CH_3COOH yield from fermented sugar is approximately 42%, with the closing sugar metabolites either lost to volatilization or transformed into different compounds[4]. Acid yield upgrades can be achieved using high rates of aeration all through continuous production. Acetobacter aceti belongs to the genus Acetobacter and hasthe potency to convert C_2H_5OH, into CH_3COOH, by a process of oxidation[5].

Vinegar historically has been used as a food preservative. whether or not clearly produced during fermentation or deliberately added, vinegar retards microbial proliferation and contributes sensory homes to a number of meals[6]. The wide diversity of products containing vinegar (sauces, ketchup, mayonnaise, etc.) and the modern fall in wine consumption have flavoured an increase in vinegar manufacturing[7]. Vinegar generally contains from 4% to 9% CH_3COOH by volume. Vinegar is commonly utilized in the cooking arts as a flavourful agent, in maintaining acidic environment ingredients, or in various pickling[8]

MATERIALS AND METHODS

2.1. WOOD APPLE ALCOHOL PRODUCTION

2.1.1. **SOURCE OF WOOD APPLE** The wood appleutilized in this current experiment was the variety '*Feronia limonia*' obtained from local markets in Delhi, India. The wood apple becomes decided on in step with the desired quality of ripeness needed at the time of procurement. Sometimes, the wood apple endured to ripen at room temperature till the right percentage of ripeness was attained.

2.1.2. PREPARATION OF WOOD APPLE JUICE

Several wood apples were selected at random to be analyzed. The whole fruits had been weighed and peeled. The wood apples had been then weighed. The wood apple components were cut with a knife, crushed with a mixer to make them juicy, sampled, and immediately frozen until analyzed. Though, the stored samples had been utilized within 72 h of collection[9].

2.1.3. PRE-TREATMENT OF WOOD APPLE SAMPLES

Moisture content in the raw wood apple samples and juicy pulp was analyzed by heat drying them in the oven at a temp of 115°C for 3 h. The titration technique of Lane and Eynonl is used to analyze the sugar content of wood apple juice and after pectinase enzyme addition, the total sugar content of wood apple juice. The wooden apples were mashed in a juicer and the juice was maintained at 15° Brixby the addition of distilled water. The pH meter evaluated the exact pH of the raw wood apple and the juicy pulp[10].

2.1.4. DEPECTINISATION

A sequential running became accomplished on the interest of the pectinase enzyme on the juicy content of wood apple which changed into very plenty bulky[11]. To acquire a clear juice from that cumbersome material, the pectinase enzyme was poured into a very minute concentration of (0.0004 %) w/v, and the wood apple juice was incubated for the timing of 10 hours at a temperature of 37ºC[12]. Optimization analysis had been performedby taking concentrations 0.0002 % (w/v), 0.0004% (w/v), 0.0006% (w/v), 0.0008% (w/v), and 0.0010 % (w/v). The excellent result was acquired at 0.0004 % (w/v) and most juice became extracted with this percent of the pectinase enzyme[13]. The 0.0002% (w/v) exhibited a final outcomeofthe incomplete breakdown of the pectin material in the juice of wood appledue toa deficiency of the enzyme required for the breakdown of the pectinaceous material[14]. The enzyme activity of the pectinase enzyme is 12 IU per gram.

2.1.5. PREPARATION OF CULTURE MEDIUM FOR INOCULUM & INOCULATION

During the preparation of the culture medium, a tweak of active Saccharomyces cerevisiae was added to the sterile water and to maintain it for 10 mins[15]. The Saccharomyces inoculum was used at 10% concentration to inoculate sterilized YEPD broth media (DW 500ml, Peptone 2.5gm, Yeast Extract 2.5gm, Dextrose sugar 5gm, pH 5). Then thisculture media was

kept for aeration at 28ºC on a rotary shaker (250rpm) for 20 hrs before the double fermentation process was accomplished[16].

2.1.7. DETERMINATION OF ALCOHOL CONTENT OF WOOD APPLE FERMENTATION BROTH

The C_2H_5OH concentrations had been anticipated for the usage of the potassium dichromate redox titrations with Sodium Thiosulphate[16].

VINEGAR PRODUCTION

Lyophilized Acetobacter aceti (MTCC 2623) turned into attained from the MTCC Gene bank, Chandigarh. The lyophilized stress become maintained on the YPM agar culture medium (Distilled Water 500ml, Yeast Extract 2.5gm, Peptone 1.5gm, Mannitol 12.5gm, Agar 6gm) slant at 37ºC for 36hrs, after which *Acetobacter aceti* way of life media became organized and prepared for inoculation to fermentation samples to synthesize vinegar[17].

ESTIMATION OF TOTAL ACIDITY % OF VINEGAR

Overall acidic content ofthe material become estimated by means of acid–the base titration approach using a well-known solution of 1 N HCl and 1 N NaOH.

The stoichiometry equation of the titration is given by:

$CH_3COOH + NaOH$-------- $CH_3COONa + H_2O$

The formula to calculate %TA as acetic acid is as beneath:

%TA = (ml of NaOH) x (N of NaOH) x (60.05) 10 x sample Weight

ESTIMATION OPTICAL DENSITY OF VINEGAR FERMENTATION BROTH Dimension of optical density in bacterial cultures is a trendy technique used in microbiology. The uses of a spectrophotometer technique to quantify the optical density at wavelength six hundred nm to observe bacterial proliferation. The OD of a bacterial manner of existence isn't an instantaneous measure of bacterial increase extensive range, but,the boom in turbidity does imply bacterial growth[18].

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

3. RESULTS AND DISCUSSION

3.1. Wood Apple ALCOHOL PRODUCTION

Samples	pH	Moisture	Brix	Total Sugar	Pectinase Addition	Total Sugar after Pectinase Addition
Raw Fruit	4.5	69.9%	-------	--------	----------	----------
Pulp	5.87	70.12%	16^{O}	19%	0.0045%	23.39%

7.8 % alcohol changed into produced via 9.01 % yeast mobile concentration at 27^{O}C, pH 3.87, and 10% sugar attention for forty-eight hrs. However, beneath the equal time of 48 hrs., 10% yeast cellular concentration produced 7.6% of alcohol. Alcohol manufacturing steadily reduced sugar concentration from around11% to 13%. Though alcohol production takes vicinity below resting situations, lower yeast cellular attention, and more quantity of sugar changed into utilized by yeast to be transformed to alcohol[19]. The distillation system turned into the maximum troublesome because it required the mixture of many pieces of gadgets, main to some malfunctions. From the above observations, we will finish that the above procedure turned into exceptionally suitable for the economic manufacturing of alcohol from wood apple end resultingin high alcohol yield and alsoenvironment-friendly method[20].

Sample	pH before fermentation	pH after fermentation	Alcohol% of fermentation broth	Residual sugar % of Fermentation Broth
Set A	3.9	3.87	7.8	0
Set B	4.3	3.87	7.6	0
Set C	4.56	3.87	6.8	0

VINEGAR PRODUCTION

PHYSICOCHEMICAL ANALYSIS

The pH, titratable acidity, and optical density are important parameters in vinegar fermentation. The end result of this look indicated that *Acetobacter acetic* with correct fermentation attributes, which may additionally decorate total acidity and limit the value of manufacturing, can be received from vinegar from wood apple alcohol. Acetic acid considers

that different organic acids are found in vinegar in negligible portions. It's far viable to think that general acidity is a superb indicator of acetic acid attention. In this observation, the physicochemical properties (pH, OD) and acetic acid content of wood apple vinegar have been analyzed. After fermentation, the vinegar produced a colorof pale brownish[21]. The results confirmed that acetic acid concentrations extended with an increase in the inoculum degree tested. The best acetic acid concentrations of 5.32 % and 5.11 % were discovered on the five and 10 % inoculum stages, respectively for the very best alcohol-contained pattern (SET C)[22]. It turned into determined that optical density or bacterial increase multiplied with an increase in inoculum degree on the same time. Acetic acid fermentation was successfully completed with the usage of wood apple juice. most of the total sugar was converted to acetic acid through ethanol. Evaporation of volatile compounds such as ethanol, acetaldehyde in addition to acetic acid at some stage in the acetic fermentation technique is one of the essential causes of lowered awareness of acetic acid[23].

Table 1: Titrable Acidity % in Vinegar Fermentation Broth

Sample Name	*Acetobacter aceti* Growth Medium Inoculation %	After 24 hrs %	After 48 hrs%	After 72 hrs %
SET A	5	4.01	5	5.11
	10	4	5	5.3
	15	3.99	4.78	5
SET B	5	4	5	5.03
	10	4.01	4.98	5
	15	4.1	4.87	5.06
SET C	5	3.07	4.89	5.11
	10	3	5.01	5.32
	15	3.17	5	5.06

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

Table 2: pH In Vinegar Fermentation Broth Measuring by pH Paper

Sample Name	*Acetobacter aceti* Growth Medium Inoculation %	After 24 hrs %	After 48 hrs%	After 72 hrs %
SET A	5	5.32	5	5
	10	5	4.9	4.89
	15	4.8	4	3.99
SET B	5	5	4.89	4
	10	5.7	5	4.9
	15	5	4.98	4.56
SET C	5	4	4	4.01
	10	4.9	4.44	4.13
	15	5	4.76	4.04

Table 3: Optical Density in Vinegar Fermentation Broth

Sample Name	Acetobacter aceti Growth Medium Inoculation %	Absorbance At 600 Nm		
		After 24 hrs %	After 48 hrs %	After 72 hrs%
SET A	5	0.36	0.4	0.6
	10	0.4	0.56	0.69
	15	0.98	0.34	0.44
SET B	5	0.99	1.02	1.2
	10	1.5	1.7	1.82
	15	1.40	1.5	1.67
SET C	5	1.01	1.2	1.6
	10	1.5	1.65	1.8
	15	1.5	1.66	1.8

Conclusion

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The outcomes of this cutting-edge experiment have verified that it is viable to generate vinegar from wooden apples. This became executed with the aid of the use of double fermentation techniques regarding *Saccharomyces cerevisiae* as a cardio stepwise degradation of sugar to ethanol and *Acetobacter aceti* oxidizers ethanol to acetic acid. The numerous parameters assessed evaluate favorably with the usual magnitudes. Furthermore, this examination encouraged that similarly, improvement of this examination by using the manner of the addition of some spices and meals additives and by using the utility of new standards located, other than extending the fermentation time and additionally searching out lines with excessive vinegar manufacturing further to appropriate tolerance closer to excessive ethanol concentrations and manufacturing temperature.

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