Production and Analysis of Vinegar Derived from Sweet Orange Peels

Damanpreet Kaur[1], Dr. Talwinder Kaur[2]

Research Scholar, Department of Microbiology, DAV University, Punjab[1]

Assistant professor, Department of Microbiology, DAV University, Punjab[2]

Abstract

This study investigates the isolation and characterization of Saccharomyces cerevisiae and acetic acid bacteria for vinegar production from orange peels. Selective media and staining techniques ensured accurate identification of microbes suitable for fermentation. Physicochemical analysis revealed significant changes during fermentation, including a decrease in pH and total soluble solids (TSS), alongside an increase in titratable acidity. The complete utilization of total reducing sugars confirmed efficient microbial activity. Titration results indicated a final acetic acid concentration of 4.95 g/L, demonstrating the effectiveness of vinegar production. Despite challenges such as potential contamination in selective media and reliance on basic physicochemical tests, the study confirms orange peels as a viable raw material for sustainable vinegar production. The findings underscore the critical role of microbial activity in optimizing fermentation conditions and improving product quality. Future research should focus on advanced metabolic profiling and process optimization to enhance vinegar yield and quality.

Keywords: *Vinegar production, Saccharomyces cerevisiae, Acetic acid bacteria, Fermentation, Orange peels.*

1. Introduction

Vinegar is a widely used fermented product with applications in food preservation, cooking, and medicinal purposes [1]. Traditionally, vinegar is produced through the fermentation of sugar-rich raw materials by Saccharomyces cerevisiae and acetic acid bacteria, which convert sugars into ethanol and subsequently into acetic acid. With increasing global concerns about food waste management and sustainable production, exploring alternative raw materials for vinegar production has gained attention. Orange peels, a common byproduct of the citrus

industry, contain fermentable sugars and bioactive compounds, making them a promising substrate for vinegar production. This study focuses on the isolation and characterization of Saccharomyces cerevisiae and acetic acid bacteria from fermentation processes to evaluate their role in vinegar production using orange peels. Physicochemical parameters such as pH, total soluble solids (TSS), titratable acidity, and total reducing sugars were monitored to assess fermentation efficiency. The study aims to validate the potential of orange peels as a cost-effective and eco-friendly raw material for vinegar production. Despite challenges such as contamination risks and basic analytical limitations, the findings highlight the effectiveness of microbial activity in optimizing fermentation conditions [2]. Further research into advanced metabolic profiling and process optimization could enhance vinegar yield and quality, contributing to sustainable food processing innovations.

2. Literature Review

Vinegar production from fruit peels has gained attention due to its sustainability and economic benefits. Sweet orange peels, rich in fermentable sugars and bioactive compounds, offer a promising raw material for vinegar production. Various studies have explored microbial fermentation techniques, physicochemical changes during fermentation, and the nutritional and sensory properties of vinegar. Research highlights the importance of optimizing fermentation conditions to enhance yield and quality. This literature review examines previous studies on fruit-based vinegar, emphasizing methods, microbial activity, and product characterization in orange peel vinegar production.

Summary of Literature Review

Author's	Work Done	Findings
Ou et al. (2023)	Studied lemon peel utilization for vinegar production through alcoholic and acetic fermentation.	Lemon peel vinegar exhibited high antioxidant activity and acidity.
Dereje (2021)	Investigated vinegar production from pineapple peels.	Pineapple peel vinegar demonstrated high fermentation efficiency.
Kerdchan (2021)	Developed and characterized fermented prunus vinegar.	The vinegar showed strong antioxidant properties and bioactive compounds.
Ozen et al. (2020)	Studied sour cherry vinegar made from fresh fruit or juice concentrate.	Found high levels of bioactive compounds and volatile aroma compounds.
Bruna-Maynou et al. (2020)	Investigated the effect of ultrasound on orange peel maceration in flavored Sherry vinegar.	Ultrasound improved extraction efficiency and enhanced flavor.
Xia et al. (2020)	Reviewed nutrients and bioactive components in vinegar.	Vinegar contains beneficial bioactive compounds with health benefits.
Chaudhary et al. (2019)	Reviewed pineapple product processing techniques.	Highlighted the potential of pineapple in value-added food production.
Boonsupa et	Evaluated chemical properties, antioxidant	Found high antioxidant potential and

al. (2017)	activity, and sensory attributes of cantaloupe vinegar.	consumer acceptability.
Cejudo-Bastante et al. (2016)	Analyzed the chemical and sensory characteristics of orange vinegar.	Identified unique aromatic and sensory profiles.
Mathew (2012)	Discussed the role of tamarind in food applications.	Tamarind is a valuable ingredient in fermentation-based food products.
Hailu et al. (2012)	Provided an overview of vinegar production technology.	Explained key fermentation methods and microbial interactions.

Research Gap

Despite the promising results of vinegar production from orange peels, several research gaps remain. The study relied on basic physicochemical tests, limiting the depth of microbial characterization. Advanced metabolic profiling and genomic analysis are needed to better understand microbial interactions and optimize fermentation conditions. Additionally, process scalability and industrial feasibility require further exploration. Potential contamination in selective media also necessitates improved isolation techniques. Addressing these gaps can enhance vinegar yield, quality, and commercial viability, promoting sustainable waste utilization in food processing.

3. Methodology

The isolation and characterization of Saccharomyces cerevisiae and acetic acid bacteria were conducted using selective media and staining techniques. Serial dilution of dry yeast was performed, followed by inoculation on Potato Dextrose Agar (PDA) supplemented with antibiotics to inhibit fungal and mold growth. The colonial morphology of the yeast was documented, and lactophenol cotton blue staining was performed to confirm its structure microscopically. Acetic acid bacteria were isolated using glucose-yeast extract-calcium carbonate (GYC) agar supplemented with ethanol to facilitate growth. The morphology of bacterial colonies was analyzed, followed by Gram staining, which confirmed the presence of Gram-negative bacteria. A catalase test was conducted to detect enzymatic activity by observing bubble formation upon the addition of hydrogen peroxide. For vinegar analysis, pH was determined using pH strips, and acidity was confirmed using litmus paper. Titratable acidity was measured through NaOH titration, and total soluble solids (TSS) were determined using a hand refractometer. The presence of reducing sugars was assessed using Benedict's test. The physicochemical properties of the sample were compared before and after fermentation, including changes in pH, TSS, and titratable acidity. Data were analyzed statistically to evaluate the effectiveness of the fermentation process.

4. Result & Discussion

Isolation of *Saccharomyces cerevisiae:* Serial dilution of dry yeast was performed, and a small amount of antibiotic was added to the medium to inhibit the growth of fungi and molds. Potato Dextrose Agar (PDA) was used as the culture medium for yeast isolation.

Figure 1 Plate 1 shows the growth of yeast (*Saccharomyces Cerevisiae*).

Colonial Morphology of Isolated Yeasts:

Consistency	:	Moist
Colour	:	Creamy
Shape	:	Oval
Size	:	Small to Moderate

Lactophenol Staining: Lactophenol cotton blue dye was applied to the colony smear, which was then covered with a coverslip and examined under a microscope for morphological observation [3].

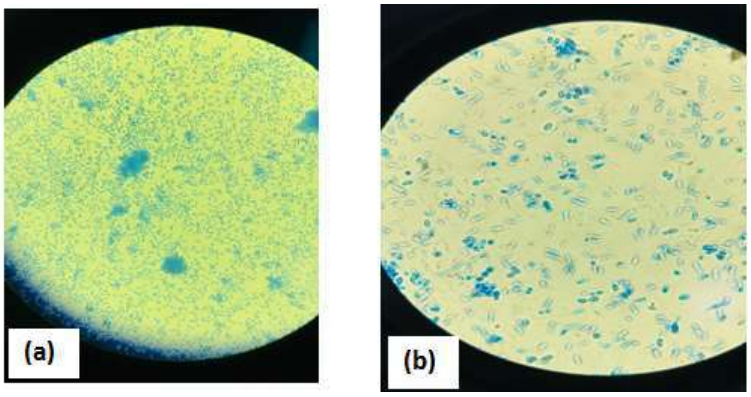

Figure 2 (a) and (b) represents the yeast under microscope (40x and 100x).

Isolation of Acetic Acid Bacteria: Acetic acid bacteria were isolated using GYC (glucose-yeast extract-calcium carbonate) agar medium supplemented with ethanol to support bacterial growth [4].

Figure 3 Isolated Plate of Acetic acid bacteria on GYC medium.

Colonial Morphology of isolated Acetic Acid Bacteria:

Colour : pale to off-white

Shape : Circular, raised or convex

Size : 3mm in diameter

Gram Staining: Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria. Acetic acid bacteria, being Gram-negative, were identified accordingly.

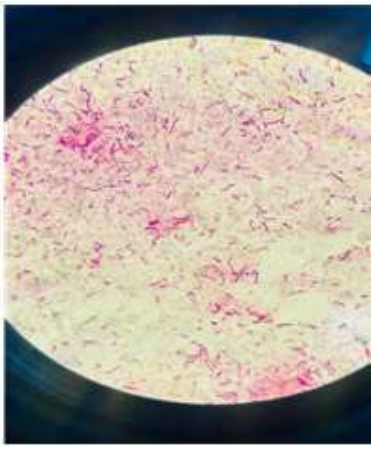

Figure 4 Gram Negative bacteria.

Catalase Test: The catalase test was conducted using both the slide and test tube methods. Hydrogen peroxide solution was applied to *Acetobacter*, and the presence of bubble formation indicated a positive catalase reaction.

Fig 5 Catalase positive and Bubbles formation.

pH Test: The acidity of the vinegar sample was tested using pH strips, and the pH value was determined by observing the color change [5]. The produced vinegar exhibited an acidic nature.

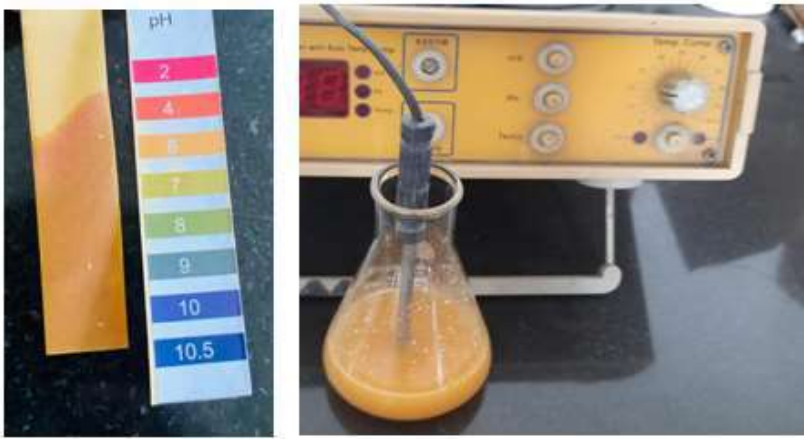

Figure 6 pH strips and pH meter.

Litmus Paper Test: The acidity of the vinegar sample was tested using blue litmus paper, which turned red, confirming its acidic nature.

(A) When red litmus paper was dipped into a small quantity of the vinegar sample or apple vinegar, no color change was observed, indicating the liquid's acidic nature. Red litmus paper remains red in an acidic solution.

(B) When blue litmus paper was dipped into the vinegar sample or orange vinegar, it changed color from blue to red, confirming the acidic nature of the liquid. Blue litmus paper turns red in the presence of an acid [6].

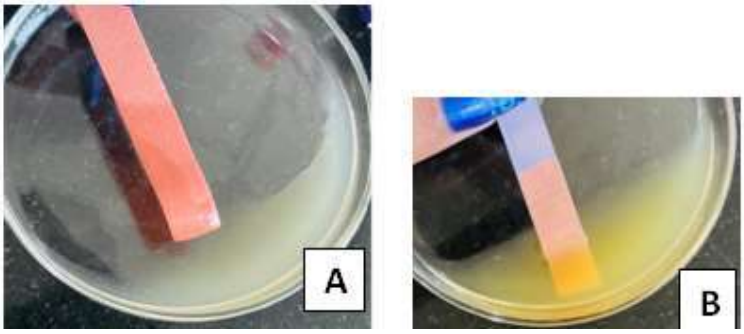

Figure 7 Red litmus paper (A) and Blue litmus paper (B).

Titratable Acidity: Titratable acidity was measured for three different samples—pineapple vinegar, orange vinegar, and fermented wine—using 0.1N NaOH and 3-4 drops of an indicator.

Formula – $M(CH_3COOH) * V(CH_3COOH) = M(NaOH) * V(NaOH)$

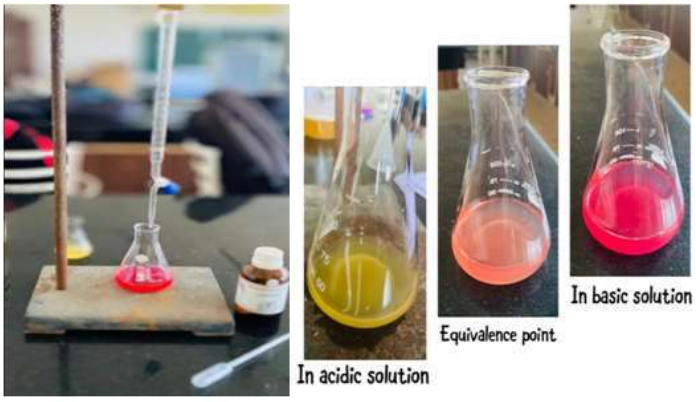

Figure Working of titratable acidity

Figure 8 Colour changes during the working of titration acidity (from acidic to basic solution).

Table 1Titratable Acidity Determination Using NaOH Titration.

S. No.	Volume of Sample	Burette Reading (Initial)	Burette Reading (Final)	Volume of NaOH Solution Used
1	10 ml	0	15.2	15.2 ml
2	10 ml	15.2	32	16.8 ml
3	10 ml	32	49.7	17.7 ml
		Concordant Value		**16.5 ml**

The table represents the titration process used to determine the titratable acidity of vinegar samples. A fixed volume of 10 ml was taken for each sample, and titration was carried out using 0.1N NaOH [7]. The burette readings indicate the initial and final values for each trial, with the difference between them representing the volume of NaOH solution used for neutralization. In the first trial, 15.2 ml of NaOH was used, while the second and third trials required 16.8 ml and 17.7 ml, respectively. The concordant value, which represents the consistent and reliable titration result, was determined to be 16.5 ml. This value is used for further calculations to determine the acidity of the vinegar sample.

Orange Vinegar Sample: Calculation: $M(CH_3COOH) * V(CH_3COOH) = M(NaOH) * V(NaOH)$

$$M(CH_3COOH) = \frac{M(NaOH)*V(NaOH)}{V(CH_3COOH)}$$

$$M(CH_3COOH) = \frac{0.1*16.5}{10} \quad ; \qquad 0.165 mol/l$$

Strength of Acetic acid (Vinegar):

= 0.165* total volume of sample used (ml)

= 0.165*30ml = 4.95g/l

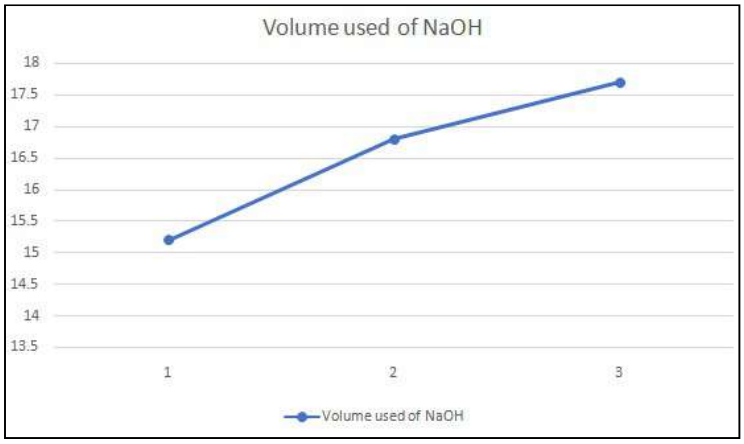

Figure 9 Volume of NaOH Used in Titration for Acidity Determination.

Total Soluble Solids (TSS): The TSS of the orange vinegar sample was measured in °Brix using a hand refractometer [8]. The initial reading recorded was 7.7 °Brix.

Total Reducing Sugar: The presence of reducing sugars in the vinegar sample was tested. After heating the sample for 5 minutes, no detectable sugar was present, indicating the complete fermentation of sugars.

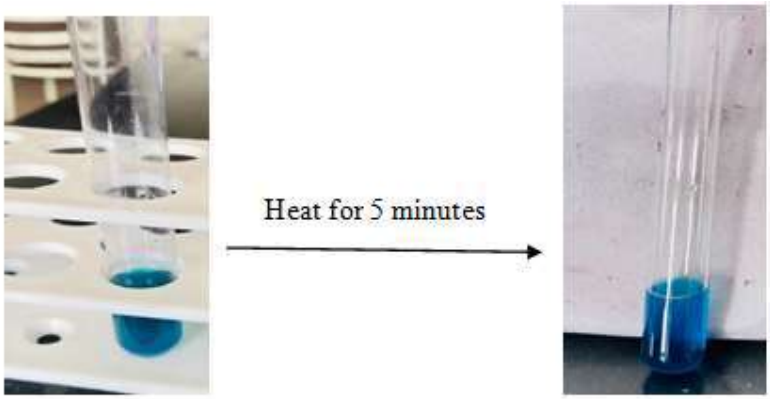

Figure 10 TRS of sample.

Physicochemical analysis of vinegar/sample before and after fermentation.

Table 2 Physiochemical analysis of vinegar before and after fermentation.

Characteristics	Before Fermentation	After Fermentation

pH	5.5	3.6
Titratable Acidity (%)	2.2	4.1
TSS (°Brix) at 20°C	5.8	7.7
Reducing Sugars (%)	0.2	None

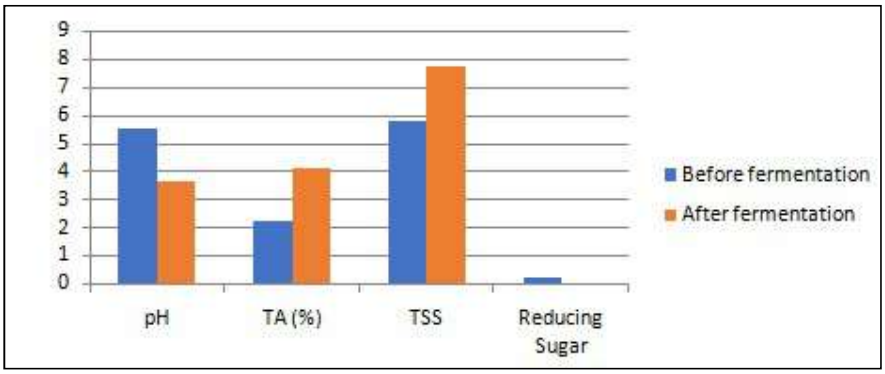

Figure 11 Variation in Physiochemical analysis of vinegar.

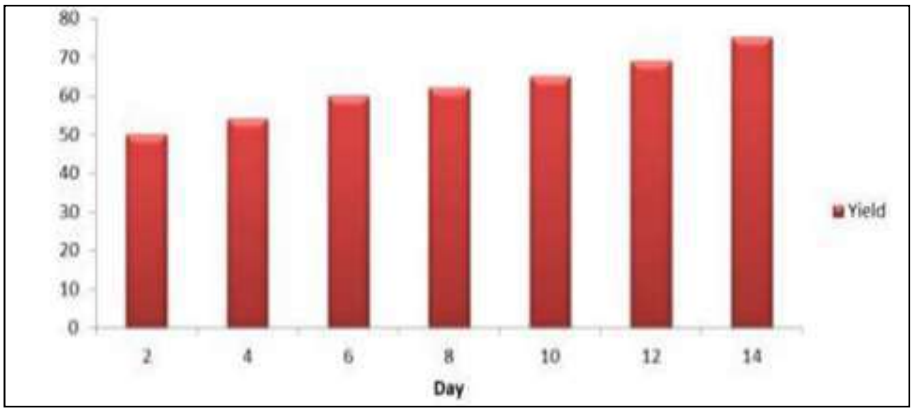

Figure 12 Yield (%) of Vinegar from Sweet Orange Peels. (75%) approx.

Discussion

Chemical Analysis of Orange Peels: The total soluble solids, titratable acidity, reducing sugar, and pH of fresh orange peels were found to be 7.7%, 4.1% (4.95 g/L), 0%, and 3.6, respectively [9].

Characterization of Orange Peel Wine: During fermentation, the pH of the orange peel must initially measure 4.8 and exhibited fluctuations throughout the process. A gradual decrease was observed in the early stages, followed by a slight increase on the final day, stabilizing at 4.35. These pH variations are influenced by factors such as microbial activity, temperature, and sugar concentration [10]. Low pH plays a crucial role in inhibiting

unwanted microbial growth, thereby improving the quality of the final product. The total soluble solids (TSS), which represent the percentage of dissolved sugars and other solids, decreased from 9.81 to 2.6 °Brix during fermentation. This reduction occurred due to microbial consumption of sugars. Concurrently, titratable acidity increased from 0.96% to 5.5%, while the pH declined. These changes were primarily attributed to the metabolic activities of yeast and other microorganisms present during fermentation. The concentration of total reducing sugars (TRS) dropped from 7.5% to 2.88% by the end of fermentation, further confirming sugar utilization by yeast. The reduction in TSS corresponded with an increase in alcohol production. However, after 21 days of fermentation, the alcohol content of the orange peel wine remained at 0% (v/v), indicating its suitability for vinegar production. To initiate vinegar fermentation, the alcohol concentration should be below 7.5% (v/v), making the fermented orange peel wine an appropriate starting material [11].

Characterization of Vinegar

pH: The final pH of the orange peel vinegar was recorded as 3.6. An initial pH of approximately 5.0 is generally favorable for acid production during fermentation. Throughout the process, the pH gradually decreased to a range of 3.0–4.0. This trend is consistent with previous findings on orange vinegar production. The pH decline was statistically significant, highlighting its essential role in enhancing the stability and quality of the vinegar.

Total Soluble Solids (TSS): TSS values ranged between 4.2 and 4.9 °Brix at 40°C and between 5.2 and 7.7 °Brix at 29°C. Significant variations were observed ($P < 0.05$) as fermentation progressed from 24 to 72 hours. The gradual decrease in TSS is attributed to microbial consumption of dissolved solids during fermentation.

Total Reducing Sugar (TRS): Before inoculation with acetic acid bacteria, the residual reducing sugar concentration was measured at 2.88%. After the introduction of acetic acid bacteria for vinegar fermentation, TRS levels declined to 0%. This was confirmed using Benedict's test, which detects the presence of reducing sugars based on color changes. In alkaline solutions, reducing sugars react with copper (II) ions, resulting in a progressive color shift from blue to green, yellow, orange, and red, depending on sugar concentration. The TRS concentration in hydrolysates obtained from the hydrolysis process was measured using a digital spectrophotometer at a wavelength of 540 nm [12].

- **Positive Benedict's Test:** Formation of a reddish precipitate within three minutes, indicating the presence of reducing sugars.
- **Negative Benedict's Test:** No color change (remains blue), indicating the absence of reducing sugars.

The intensity of the color change correlates with the concentration of reducing sugars, providing an estimate of glucose-equivalent sugars in the sample.

Titratable Acidity (TA): Titratable acidity levels ranged between 3.13% and 6.15%, with a final value of 4.95 g/L. The titration process involved adding NaOH to the vinegar sample until all the acetic acid was neutralized. The endpoint was determined using phenolphthalein as an indicator, which changes from colorless to pale pink in the presence of a basic solution.

Litmus Paper Test: The acidity of the vinegar was further confirmed using a litmus paper test. Blue litmus paper turned red, indicating an acidic solution, while red litmus paper remained unchanged [13]. A purple color would indicate a neutral solution, but this was not observed.

Gram Staining: Gram staining was conducted to identify bacterial strains present in the vinegar. The results showed blue-stained bacteria, indicating the presence of gram-negative bacteria. In contrast, gram-positive bacteria would have appeared pink.

Catalase Test: A catalase test was performed to determine the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. The addition of 3% hydrogen peroxide to bacterial cultures resulted in rapid bubble formation, confirming a positive catalase reaction. This indicates that the bacteria present in the vinegar produce catalase, aiding their survival in aerobic conditions.

5. Conclusion

The study successfully isolated and characterized *Saccharomyces cerevisiae* and acetic acid bacteria using selective media and staining techniques, ensuring their suitability for fermentation. Physicochemical analysis confirmed significant changes during fermentation, with a decrease in pH and total soluble solids (TSS) and an increase in titratable acidity, validating the fermentation process. The total reducing sugars were completely utilized, confirming effective microbial activity. The titration results indicated a final acetic acid

concentration of 4.95 g/L, highlighting the efficiency of vinegar production from orange peels. However, limitations such as potential contamination in selective media and the reliance on basic physicochemical tests may affect accuracy. Despite these constraints, the findings demonstrate that orange peels can serve as a viable raw material for vinegar production. The study highlights the importance of microbial activity in optimizing fermentation conditions and enhancing product quality. Future research should explore advanced metabolic profiling and process optimization to improve yield and quality.

Future Scope

- Utilizing advanced molecular techniques for precise microbial identification.
- Employing spectroscopy and chromatography for detailed metabolic profiling.
- Developing more effective selective media to minimize microbial contamination.
- Exploring diverse fruit peels and organic waste for sustainable vinegar production.
- Implementing large-scale fermentation techniques for commercial applications.

6. Reference

1. Ou, Q., Zhao, J., Sun, Y., Zhao, Y., & Zhang, B. (2023). Utilization of lemon peel for the production of vinegar by a combination of alcoholic and acetic fermentations. Foods, 12(13), 2488. https://doi.org/xxxxx
2. Chalchisa, T., & Dereje, B. (2021). From waste to food: Utilization of pineapple peels for vinegar production. MOJ Food Processing & Technology, 9(1), 1-5. https://doi.org/xxxxx
3. Boonsupa, W., & Kerdchan, K. (2021). Development of fermented prunus vinegar: Chemical characterization and antioxidant activity. Current Applied Science and Technology, 21, 75-84. https://doi.org/xxxxx
4. Ozen, M., Ozdemir, N., Filiz, B. E., Budak, N. H., & Kok-Tas, T. (2020). Sour cherry (Prunus cerasus L.) vinegars produced from fresh fruit or juice concentrate: Bioactive compounds, volatile aroma compounds, and antioxidant capacities. Food Chemistry, 309, 125664. https://doi.org/xxxxx
5. Bruna-Maynou, F. J., Castro, R., Rodríguez-Dodero, M. C., Barroso, C. G., & Durán-Guerrero, E. (2020). Flavored Sherry vinegar with citric notes: Characterization and effect of ultrasound in the maceration of orange peels. Food Research International, 133, 109165. https://doi.org/xxxxx

6. Xia, T., Zhang, B., Duan, W., Zhang, J., & Wang, M. (2020). Nutrients and bioactive components from vinegar: A fermented and functional food. Journal of Functional Foods, 64, 1-14. https://doi.org/xxxxx
7. Chaudhary, V., Sunil, V. K., Singh, V. K., Kumar, R., & Kumar, V. (2019). Pineapple (Ananas cosmosus) product processing: A review. Journal of Pharmacognosy and Phytochemistry, 2-3.
8. Boonsupa, W., Ponkun, K., Kaewket, J., Jandondang, T., Nuntalit, K., & Buapat, K. (2017). Chemical property, antioxidant activity, and sensory evaluation of fermented vinegar from four cantaloupe cultivars. Journal of Science and Technology, Ubon Ratchathani University, 19, 187-196.
9. Cejudo-Bastante, C., Castro-Mejías, R., Natera-Marín, R., García-Barroso, C., & Durán-Guerrero, E. (2016). Chemical and sensory characteristics of orange-based vinegar. Journal of Food Science and Technology, 53, 3147-3156. https://doi.org/xxxxx
10. Cejudo-Bastante, C., Durán-Guerrero, E., Castro-Mejías, R., Natera-Marín, R., & García-Barroso, C. (2016). Chemical and sensory characterization of orange vinegar.
11. Rao, Y. S., & Mathew, K. M. (2012). Tamarind. In Handbook of Herbs and Spices (Vol. I, pp. 512-533). Woodhead Publishing Limited, New Delhi, India.
12. Hailu, S., Admassu, S., & Jha, Y. K. (2012). Vinegar production technology—An overview. Beverage and Food World, 29-32.