

#### ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume X Issue VI June 2025

# Biotechnological Studies on the Fermentation and Physicochemical Characterization of Palm Fruit, Tiger Nut, and Watermelon Juices for Wine Production

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DOI: https://doi.org/10.51584/IJRIAS.2025.10060076

Received: 02 June 2025; Accepted: 06 June 2025; Published: 11 July 2025

## **ABSTRACT**

This study investigated the fermentation potential of juice blends derived from palm fruit (Phoenix dactylifera), tiger nut (Cyperus esculentus), and watermelon (Citrullus lanatus) for wine production. The formulation involved blending different volumes of juice from the fruits, with the addition of a sugar solution, Saccharomyces cerevisiae, and nutrients (ammonium phosphate and potassium phosphate) to support the fermentation process. Primary fermentation was conducted over six days in airtight containers. This was followed by a secondary fermentation phase lasting fourteen days under anaerobic conditions. The results showed that during primary fermentation, wine samples exhibited notable fluctuations in temperature and pH. Temperature readings across all samples peaked on day three, ranging from 29.0°C to 30.2°C, before declining to 26.1°C to 27.5°C by day six. Single-matrix juices displayed slightly lower initial temperatures (26.4°C to 27.0°C) compared to blended juices, which started between 28.0°C and 28.2°C. The pH values consistently decreased over the six-day fermentation period, from an initial range of 3.74 to 4.80 to a final range of 3.00 to 3.13, with blended juices showing a more pronounced pH drop (4.80 to 3.13) than single-matrix juices (3.74 to 3.01). After secondary fermentation and clarification, the final temperatures of the wine samples ranged from 27.0°C to 28.1°C. Specific gravity values ranged between 0.8932 and 1.0002, indicating near-complete sugar utilization. The final pH values were between 2.88 and 3.89, alcohol content varied from 14.63% to 15.66%, and total titratable acidity ranged from 0.54% to 0.65%. This study concluded that all wine samples underwent a successful fermentation and all exhibited physicochemical properties which are within the standard of commercially acceptable wines, with wine gotten from date palm fruit and watermelon having the most balanced physicochemical profile. Recommendation included adopting the insight from this study to remedy the excessive wastage of fruit around the world.

**Key words:** Fermentation potential, juice blends, palm fruit, tiger nut, watermelon, wine production.

## INTRODUCTION

The global challenge of excessive food wastage, particularly in developing nations, has become a significant concern in recent years. According to the Food and Agriculture Organization (FAO), approximately one-third of food produced for human consumption is lost or wasted globally each year, with fruits being a major contributor [1]. This widespread waste presents not only environmental and economic challenges but also hinders efforts to address food insecurity in resource-limited settings. One potential solution to mitigate fruit wastage is the transformation of excess fruits into value-added products, which can offer both economic and nutritional benefits [2, 3]. Wine production from non-traditional fruits offers a novel and sustainable approach to utilizing these underutilized resources while enhancing food security.

In conventional winemaking, grapes are the primary raw material due to their favourable sugar content and fermentation properties. However, the increasing availability of alternative fruits, such as tiger nut, palm fruit, and watermelon, has sparked interest in exploring their potential for fermentation and wine production. These fruits are rich in sugars, vitamins, and minerals, making them ideal candidates for fermentation processes that



ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume X Issue VI June 2025

can yield high-quality wines with distinct flavors and nutritional properties. Palm fruit is widely consumed across various regions in Africa and Asia and is valued for its diverse applications. However, post-harvest losses and inefficiencies in processing can still lead to significant quantities being discarded [4]. Similarly, watermelon and tiger nut have demonstrated potential as raw materials for fermentation, contributing to both the reduction of food waste and the production of a novel alcoholic beverage [5, 6].

Fermentation is a biotechnological process by which microorganisms such as yeast convert sugars into alcohol and other byproducts [7]. The efficiency of fermentation is influenced by factors such as fruit composition, yeast strain selection, fermentation conditions, and the presence of various sugars and organic acids. As a method for producing alcoholic beverages and other foods, fermentation is widely regarded as safe when properly managed, offering unique advantages over other food processing techniques like thermal processing, chemical preservation, or irradiation. According to Anumudu et al. [8], fermentation enhances food safety by creating an acidic environment that inhibits pathogenic bacteria, while also improving nutritional value and flavor, making it a critical and reliable technique in biotechnology.

While alternative wine production methods are gaining attention, the use of blended juices for wine production is an emerging area of research. The potential benefits of such a process include the reduction of food waste, the creation of new flavors, and the development of economically viable products for both local consumption and export. However, questions remain about the fermentation dynamics, the interplay of sugars and acids, and how different fruit blends may influence the final wine's physicochemical properties. Thus, this study explored the fermentation process, the effect of blending on wine characteristics, and the potential for large-scale adoption of these fruit-based wines. This study aims to contribute to the broader field of sustainable winemaking practices and highlights the role of biotechnology in transforming underutilized fruits into valuable, marketable products.

## MATERIALS AND METHODS

#### **Materials**

The materials and apparatus used for the study included: Palm fruit (Phoenix dactylifera), Tiger nut (Cyperus esculentus), Watermelon (Citrullus lanatus), Distilled water, Ammonium phosphate, Potassium phosphate, Saccharomyces cerevisiae (baker's yeast), White transparent plastic buckets, Electric blender

## **Collection of Samples**

Fresh samples of palm fruit, tiger nut, and watermelon were purchased from Ogbe-Hausa Market, located in Abakaliki, Ebonyi State, Nigeria.

## **Preparation of Fruit Juices**

Each of the fruits was washed thoroughly to remove dirt and debris. The watermelon was peeled to obtain the fleshy portion, and the seeds were removed along with those of the palm fruit. Each fruit was blended separately using an electric blender, with 150 cm³ of clean distilled water added to aid the blending process. The blended fruit pulp was filtered using a clean muslin cloth to extract the juice. Wine was produced from both single fruit juices and blended combinations.

### **Blending of Fruit Juices**

Fruit juices were blended following the procedure outlined by Ohoke and Nwokonkwo[9]. Seven different wine samples were prepared: four from blends of two or more fruit juices and three from single fruit sources. The composition and labeling of each wine sample are shown in Table 1.

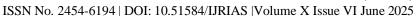




Table 1: Composition and Labeling of Wine Samples

Wine Sample	Juice Composition (Volume)	Fruit Combination
Wine A	Palm fruit: 3150 cm <sup>3</sup> + Watermelon: 3150 cm <sup>3</sup>	Palm fruit and Watermelon
Wine B	Palm fruit: 3150 cm <sup>3</sup> + Tiger nut: 3150 cm <sup>3</sup>	Palm fruit and Tiger nut
Wine C	Watermelon: 3150 cm <sup>3</sup> + Tiger nut: 3150 cm <sup>3</sup>	Watermelon and Tiger nut
Wine D	Palm fruit: 2100 cm <sup>3</sup> + Watermelon: 2100 cm <sup>3</sup> + Tiger nut: 2100 cm <sup>3</sup>	All three fruits blended
Wine E	6300 cm <sup>3</sup> (Palm fruit only)	Palm fruit only
Wine F	6300 cm <sup>3</sup> (Tiger nut only)	Tiger nut only
Wine G	6300 cm <sup>3</sup> (Watermelon only)	Watermelon only

Each juice or juice blend was transferred into a clean transparent plastic bucket and allowed to stand for three hours. A sugar solution (200 g of sugar in 70 cm³ of water) was prepared and added to each sample. Subsequently, 0.90 g of Saccharomyces cerevisiae, 0.60 g of ammonium phosphate, and 0.60 g of potassium phosphate were added to support fermentation, as described by Ohoke and Nwokonkwo [9].

#### **METHODS**

## **Primary Fermentation**

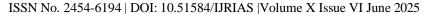
Primary fermentation was conducted in airtight transparent plastic containers over six days. The must was stirred vigorously every 12 hours. During fermentation, parameters such as temperature, pH, specific gravity, total titratable acidity, and sugar level (°Brix) were measured at regular intervals.

## **Secondary Fermentation**

After six days, the wine was racked into a secondary fermenter, which was an airtight container equipped with a fermentation lock. A rubber tube connected the fermenter to a bucket containing clean water. Fermentation was monitored by the presence of bubbles passing through the water. This secondary fermentation lasted 14 days and was considered complete when no bubbles were observed, indicating the cessation of gas release [9].

Upon completion, the wine was clarified using bentonite as described by Ogodo et al. [10]. To prepare the bentonite solution, 125 g of bentonite was dissolved in 500 cm³ of boiling water and stirred until a gel was formed. The solution was allowed to stand for 24 hours, after which 40 g of the gel was added to each wine sample. The mixture was stirred thoroughly to ensure uniform distribution. Clarification was monitored using a tightly sealed bottle sample. After one month, the clarified wine was filtered through a muslin cloth and fine mesh sieve.

The Fermentation process was carried in two stages because primary fermentation under aerobic conditions is necessary for robust yeast growth and efficient sugar breakdown, while secondary fermentation under anaerobic conditions is essential to complete alcohol production, prevent oxidation, and enhance clarity, stability, and flavor.





## **Total Titrable Acidity of the Wine**

The total titratable acidity was determined using the method outlined by Ogu and Mgbebu [11]. Ten millilitres of wine were measured into a conical flask, and phenolphthalein was added as an indicator. The solution was titrated against 0.1 N sodium hydroxide (NaOH). The titratable acidity was calculated using the formula:

Total titrable acidity (TTA) =  $\frac{\text{V1} \times \text{N} \times 75}{1000 \times \text{V}}$ X 100

Where;  $V_1 = \text{Volume (cm}^3) \text{ of NaOH}$ 

 $V = Volume (cm^3)$  of sample used

N = Normality of NaOH

## pH and Temperature of the Wine

The pH and temperature were also determined using a calibrated digital (HANNA) pH meter and an analytical thermometer respectively.

## **Specific gravity**

The specific gravities of the wine were determined using the hydrometer

#### **Alcohol content**

The alcohol content of the wine was determined using psycnometer obtained from the CAS, Ebonyi state Campus. The psycnometer employs Archimedes' principle of fluid displacement and Boyle's law of volume-pressure relationships, respectively, for liquid and gas pycnometers. The results were calculated as follows:

Percentage alcohol =  $(OG - FG) \times 0.575\%$ 

Where, OG = Original Gravity of the sample

FG = Final Gravity of the sample

#### RESULTS

## **Temperature Variations during Primary Fermentation**

Table 2 shows the changes in temperature observed over six days of primary fermentation for each wine sample. Temperature readings were recorded daily. A general fluctuation was noted across all samples, with initial increases followed by gradual decreases, indicating active microbial metabolism and subsequent stabilization.

Table 2. Temperature (°C) variations of wine samples during primary fermentation

Time (Days)	Wine A	Wine B	Wine C	Wine D	Wine E	Wine F	Wine G
1	28.2	28.0	28.1	28.0	27.0	26.4	26.9
2	28.7	29.0	28.8	29.0	28.7	27.5	28.0
3	29.0	30.1	30.2	29.5	29.0	28.9	29.4
4	28.3	30.0	29.2	28.9	28.3	27.5	28.9

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume X Issue VI June 2025



5	27.0	27.0	28.0	28.0	27.5	27.0	28.4
6	27.5	26.1	27.0	26.8	27.0	26.8	27.3

## pH Variations during Primary Fermentation

Table 3 presents the pH progression of the fermenting wine samples across six days. A consistent decline in pH was observed, indicative of organic acid production during fermentation. This trend is characteristic of yeast activity and microbial breakdown of fermentable sugars.

Table 3. pH variations of wine samples during primary fermentation

Time (Days)	Wine A	Wine B	Wine C	Wine D	Wine E	Wine F	Wine G
1	4.25	4.20	4.51	4.80	3.88	3.74	3.90
2	3.80	4.00	4.02	4.20	3.75	3.66	3.79
3	3.61	3.59	3.82	3.75	3.70	3.60	3.61
4	3.26	3.25	3.40	3.53	3.55	3.40	3.50
5	3.16	3.11	3.10	3.25	3.40	3.10	3.25
6	3.10	3.01	3.00	3.13	3.00	3.01	3.10

## **Final Physiochemical Properties of the Wines**

Table 4 summarizes the final physiochemical characteristics of the wines after the completion of fermentation and clarification. These parameters include temperature, specific gravity, pH, alcohol content, and total titratable acidity. The alcohol content across all samples ranged from 14.63% to 15.66%, reflecting efficient fermentation. Specific gravity values suggest complete sugar utilization in most blends.

Table 4. Physiochemical properties of the final wine samples after secondary fermentation

Wine Sample	Temperature (°C)	Specific ravity	рН	Alcohol Content (%)	Total Titratable Acidity (%)
A	27.90	1.0000	3.72	15.66	0.61
В	27.80	0.9111	3.88	15.03	0.65
C	28.10	0.8932	3.70	14.63	0.58
D	28.00	0.9932	3.89	14.99	0.63
E	27.50	0.9991	2.88	15.00	0.55
F	27.20	1.0002	2.95	15.00	0.54
G	27.00	0.8999	3.00	14.88	0.63



## **DISCUSSION**

## **Temperature**

The observed temperature variations during primary fermentation reflect the metabolic activity of yeast and other fermentative microorganisms. All wine samples exhibited an initial rise in temperature between day 1 and day 3, followed by a general decline from day 4 to day 6. This pattern suggests a peak in fermentation activity around day three, a stage commonly associated with the exponential growth phase of yeast cells [12, 13]. Wine C and Wine B recorded the highest temperatures at day three, reaching 30.2°C and 30.1°C respectively. The gradual decline in temperature after the third day is likely a consequence of reduced sugar availability and the accumulation of ethanol, both of which can inhibit further microbial activity [14]. It is noteworthy that Wine F and Wine G maintained slightly lower temperature values throughout the fermentation process (Figure 1). This could be attributed to their initial sugar concentrations or the influence of their respective fruit matrices, which may have affected microbial growth dynamics [15, 16].

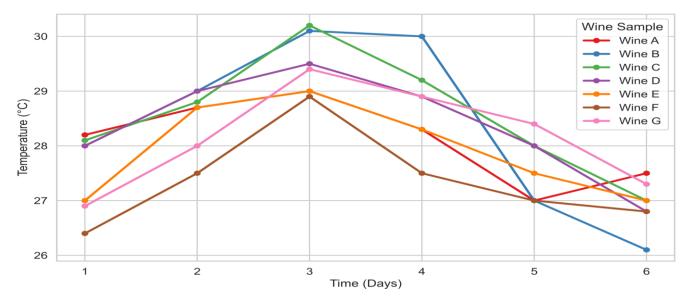


Figure 1. Temperature variations of the wine samples during primary fermentation.

Temperature is an important parameter of wine fermentation, as it determines yeast performance, aroma retention, and overall fermentation kinetics. According to the International Organisation of Vine and Wine (IOV), standard fermentation temperatures for fruit wines should range between 20°C and 30°C. Values beyond this range may risk compromising flavour or arresting yeast activity [9]. As seen in this study, all wine samples recorded temperatures within this standard, with peak values of 30.2°C and 30.1°C in Wine C and Wine B respectively (Figure 1). These values reflect a vigorous yet controlled fermentation process, consistent with what is expected during the active metabolic phase of Saccharomyces cerevisiae. Final temperatures (after the secondary fermentation) of the wine samples ranged between 27.00°C and 28.10°C, which are slightly lower than the peak values observed during primary fermentation. This decline is typical as metabolic activity reduces in the later stages of fermentation [16].

#### рH

pH values across all wine samples showed a consistent decline over the six-day fermentation period. This acidification is characteristic of fermentative metabolism, particularly the production of organic acids such as tartaric, malic, and acetic acids by yeast and associated microbial flora [15]. The most significant pH reduction was observed in Wine D, which dropped from 4.80 on day one to 3.13 on day six of the primary fermentation (Figure 2). Although Wine E and Wine F started at lower initial pH values compared to others, they still followed the same downward trajectory.



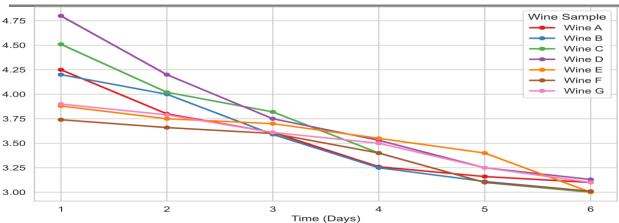


Figure 2. pH variations of the wines samples during primary fermentation.

In wine fermentation, pH influences microbial stability, colour, and the overall chemical profile [17]. Standard wine pH values generally fall between 3.0 and 3.8, depending on the fruit type and winemaking technique. According to the OIV and Codex Alimentarius, values below 3.0 may increase tartness and suppress spoilage organisms, while values above 4.0 are discouraged due to instability risks. Post-fermentation pH values varied from 2.88 to 3.89 across the wine samples (Table 3). Wines E, F, and G exhibited lower pH values (2.88–3.00), indicating higher acidity and potentially enhanced microbial stability and shelf life. In contrast, samples A to D maintained moderately higher pH values (3.70–3.89), which could influence the taste profile and fermentation completeness (Figure 2). The overall pH decline from primary to secondary fermentation supports the production of organic acids during microbial activity [13, 16].

#### **Alcohol Content**

In terms of final physicochemical properties, alcohol content ranged from 14.63% to 15.66%, indicating successful fermentation and adequate sugar conversion. Wine A showed the highest alcohol content (15.66%), which may suggest a higher initial sugar concentration or enhanced yeast performance under the fermentation conditions applied. These values are comparable to those reported in previous studies on naturally fermented tropical wines [18], and they meet the general alcohol content standard for commercially acceptable wines.

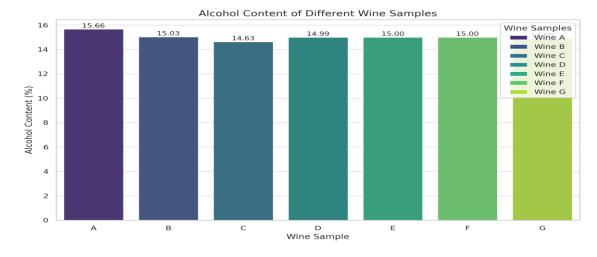


Figure 3: Alcohol Content (%) of Wine Samples

#### **Specific Gravity**

Specific gravity is a direct indicator of sugar conversion during wine fermentation. According to industry practice, a final specific gravity between 0.9900 and 1.0000 suggests a dry wine with minimal residual sugars [17]. Fermentations that reach values below 0.9900 are often considered complete or overextended, depending on the context [13].



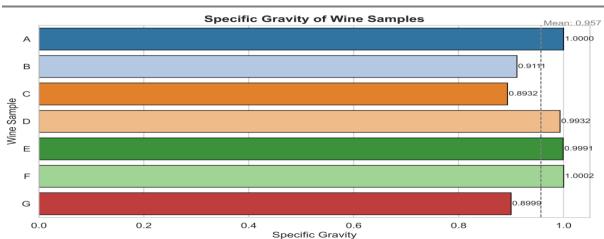


Figure 4: Specific Gravity of Wine Samples

As seen in this study, Wines B and C achieved final specific gravities of 0.9111 and 0.8932, respectively. B and Wine C had the lowest final specific gravity values (0.9111 and 0.8932 respectively), suggesting near-complete fermentation (Figure 4). These wines also had relatively high alcohol levels, which aligns with the expected inverse relationship between residual sugar (as indicated by specific gravity) and ethanol production.

## The total titratable acidity (TTA

The total titratable acidity (TTA) of the final wines fell between 0.54% and 0.65%. These values are within the typical range for fruit-based wines and contribute to taste balance, microbial stability, and preservation [15]. Wine B recorded the highest TTA at 0.65%, which may correlate with its higher pH and moderate alcohol level. Wines E and F had the lowest TTA values, which could affect their overall flavor complexity (Figure 5). However, in combination with their lower pH values, they may offer longer shelf stability.

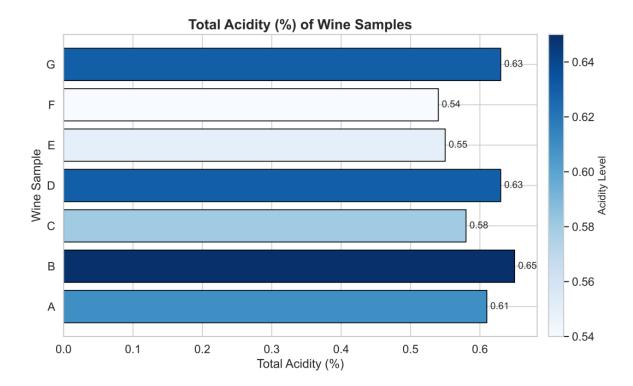
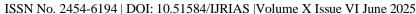


Figure 5: Total Acidity (%) of Wine Samples

Standard TTA values for fruit wines, expressed as tartaric acid equivalent, usually range from 0.5% to 0.9%. According to the Food and Agriculture Organization and various oenological studies, values below 0.5% may





result in flatness, while those above 1.0% can lead to harshness [17]. As seen in this study, all samples recorded TTA values within the acceptable range (figure 5). These results are consistent with the acidity profiles of tropical wines reported in related studies from sub-Saharan Africa and Southeast Asia [18]. The slight differences across wines reflect fruit-specific organic acid profiles and confirm that acid development during fermentation remained under control [15]. The earlier fermentation pattern, involving aerobic primary and anaerobic secondary phases, likely influenced pH trends by promoting initial acid production, followed by stabilization as yeast metabolism shifted under reduced oxygen conditions [19].

## Physiochemical Profile of Blended vs Single-Fruit Wine

There was a notable impact of the fruit matrix on the fermentation dynamics of each wine. Wines A–D, which were formulated from combinations of blended fruits, generally demonstrated higher alcohol content and titratable acidity compared to their single-fruit counterparts. For instance, Wine A recorded the highest alcohol content at 15.66%, and Wine B had the highest TTA at 0.65%. These values suggest that fruit blending enhanced the fermentable sugar load and possibly created a more favourable environment for yeast metabolism. On the other hand, single-fruit wines such as Wine E and Wine F, although slightly lower in alcohol and acidity, exhibited lower pH values (2.88 and 2.93 respectively), which may improve microbial stability and preservation. The lower specific gravity values observed in some single-fruit wines indicate that sugar consumption was still effective, though perhaps moderated by the fruit's intrinsic composition. These observations align with existing reports that support the blending of fruits in winemaking to improve nutrient balance, optimize fermentation kinetics, and enhance the sensory and stability profiles of the final product [15, 18].

## **CONCLUSION**

This study successfully explored the fermentation performance and physicochemical characteristics of wines produced from individual and blended juices of palm fruit (Phoenix dactylifera), tiger nut (Cyperus esculentus), and watermelon (Citrullus lanatus). The findings demonstrate that blending these fruits enhanced fermentation efficiency, resulting in higher alcohol yield, improved sugar conversion, and balanced acidity profiles. The highest alcohol content (15.66%) was recorded in wine gotten by mixing palm fruit and watermelon (3150cm<sup>3</sup>:3150cm<sup>3</sup>), while others exhibited optimal fermentation kinetics.

In contrast, wines produced from single fruit substrates showed comparatively lower alcohol and titratable acidity but maintained desirable low pH values that are favourable for microbial stability and preservation. These differences underscore the role of fruit matrix composition in shaping fermentation dynamics and final product quality. Blended formulations appear to provide a more favourable nutrient environment, improving yeast performance and resulting in more robust fermentation outcomes.

In overall, the results support the feasibility of producing stable, naturally fermented wines from these tropical fruit combinations, and provides valuable insights into non-conventional fruit winemaking, with implications for commercial development, particularly in regions where these fruits are readily available.

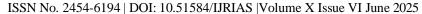
## Limitations

A key limitation of this study is the absence of a sugar-only control fermentation. This would have served as a standard to better evaluate the fermentation efficiency and alcohol yield of the fruit-based musts. Time constraints and logistical challenges prevented the inclusion of this comparative experiment in the current study.

#### **List of Abbreviations**

FAO Food and Agriculture Organization

NaOH Sodium hydroxide





IOVInternational Organisation of Vine and Wine

TTATotal titrable acidity

#### **Author Contributions**

A.H.A: Investigation, Resources, Supervision, Funding acquisition, Writing—review & editing. A.H.A., V.O.U., and C.B.N.: Methodology, Data curation, Visualization, Formal analysis, Writing—origina, draft. A.H.A.: Writing—review & editing,. A.H. A: Sample collection. A.H.A: Supervision. A.H.A., V.O.U., C.B.N., C.N.O., M.C.C., I.C.V., and I.K.U: Resources. A.H.A: Funding acquisition, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

## **Availability of Data and Materials**

The data that support the findings of this study are available on request from the corresponding author.

#### **Consent for Publication**

The authors give the publisher the permission of the author to publish the work.

#### **Conflicts of Interest**

The authors declare no conflicts of interest regarding this manuscript.

## **Funding**

This research project did not get any external funding.

## Acknowledgments

Declared none

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