

# Effect of Fermentation on the Proximate Composition and Physicochemical Properties of Blends of Watermelon and Tigernut Juice

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## Abstract

Tigernut tuber and Watermelon fruit are common substrates found in Nigeria. This study analyzed the proximate and physicochemical properties in a consortia of fermenting Tigernut and Watermelon blends. The substrates used were processed and evenly divided into three sterile fermenters each containing three litres of the blended substrates. The substrates was allowed to undergo spontaneous fermentation via the action of wild yeast (Chance inoculation) for 5 days (primary fermentation) after which the mixtures were sieved with a muslin cloth and transferred into a secondary fermenter and allowed to ferment for 4 weeks. The fermented samples had an initial pH of  $6.51 \pm 0.00$ ,  $6.43 \pm 0.00$ ,  $6.67 \pm 0.00$  which later got reduced at the end of the fermentation to  $3.15 \pm 0.00$ ,  $3.16 \pm 0.00$ ,  $3.22 \pm 0.00$  in Samples A, B and C respectively. The Total Titrable Acid increased from  $0.49 \pm 0.02 \text{g/L}$  to  $0.96 \pm 0.13 \text{g/L}$ ,  $0.34 \pm 0.02 \text{g/L}$  to  $1.52 \pm 0.24 \text{g/L}$ ,  $0.40 \pm 0.07 \text{g/L}$  to  $1.45 \pm 0.29 \text{g/L}$  for samples A, B and C respectively. For the proximate composition of the substrates, The Ash content reduced from an initial value of 0.70 to 0.48%, 0.80 to 0.44% for samples A and C but got increased in sample B with value of 0.36 to 0.37%. The crude Fat reduced from 1.53 to 0.46%, 1.30 to 0.30% and 2.17 to 0.26% for samples A, B and C respectively. The crude Protein reduced from 2.21 to 1.31%, and 2.18 to 2.06% for samples A and C respectively but got increased in sample B with values of 1.64% to 1.88%. While the total carbohydrate reduced from an initial 7.09 to 0.74%, 4.16 to 0.00% and 13.67 to 0.00% for samples A, B and C respectively. This study shows that the resulting product had low fat content which could provide protection against excess body lipids.

## I. INTRODUCTION

Tigernut (*Cyperus esculentus*), is a perennial crop that is widely cultivated in the Arabian Peninsula, East Africa, Spain, Asia, and East Africa. It belongs to the *Cyperaceae* family and is also known as chufa, yellow nuts, earth almond, and ground almond. (Abdelkader *et al.*, 2017). There are three prevalent types of this nut in Nigeria: brown, yellow, and black, albeit the latter two are the easiest to get in stores (Adenugba *et al.*, 2024). Due to its attractiveness, high milk yield, fleshier body, low fat content, high protein content, and fewer anti-nutritional elements, the yellow chufa is preferred among the other two types (Ponnampalam, *et al.*, 2024). This tuber can be eaten as a snack, roasted, fried, or baked in addition to being used to make flour, starch, cakes, and biscuits. These tasty tubers are generally soaked in water before consumption and have a sweet nutty flavor. It can also smell like ice cream and cookies due to its distinct sweetness (Zhang and Sun, 2023). According to Gelberg (2017), tiger nuts have been referred to as "health" foods since they are believed to increase blood circulation, lower the risk of colon cancer, and shield against heart disease and thrombosis when taken.

Also, indigestion, debility, diarrhoea, dysentery, and flatulence can all be treated with tiger nuts (Gelberg, 2017). Tiger nut tubers have around twice as much starch as potatoes or sweet potatoes. Additionally, it is used as a flavoring and nutritional supplement, as well as in the production of the local beverage kunnu, as well as in the making of nougat, jam, beer, ice cream, and jam (Yu *et al.*, 2022). It contains a lot of high-quality oil that may be fried in or naturally used with salads. tiger nut "milk" has been researched as a milk substitute for fermented foods like yoghurt manufacturing and in the diets of those who are lactose intolerant (Oke *et al.*, 2019).

Watermelon (*Citrullus lanatus*) is a fruit that belongs to the *Cucurbitaceae* family. The quantity and proportion of species in the *Cucurbitaceae* family that are used as food by humans is among the highest of all plant groups. It is a big, spreading annual plant with yellow blooms and rough, hairy leaves with pinnate lobes cultivated for its edible fruit, a unique variety of berry known botanically as a pepo (Paris, 2015). The watermelon fruit has a thick, deep green skin that is smooth and striped vertically in grey or light green. Small black seeds are inserted in the middle of the

fruit's center third of crimson flesh (Nadeem *et al.*, 2022). Depending on how it is made, watermelon can be fermented, mixed, and consumed in Nigeria as juice, nectars, fruit cocktails, as well as an appetizer or snack. Fruits like watermelon and tiger nuts, for example, are among the most essential nutrients for humans as they are not only nutritional but also essential for maintaining good health (Nadeem *et al.*, 2022).

Fresh and processed fruits both enhance the nutritional value of our diet and supply necessary nutrients like vitamins, minerals, carbs, etc. Fresh fruit postharvest loss is one of the major issues facing tropical nations like India. Nearly 35 to 40 percent of horticultural yield is wasted as a result of inappropriate postharvest management and limited processing facilities, hence the need to preserve these nutrients through fermentation (Bhardwaj *et al.*, 2024).

The aim of this study is to investigate the effect of fermentation on proximate composition and physicochemical properties (pH, titratable acidity) of these blends of fruit over the course of four weeks.

## II. MATERIALS AND METHODS

Table 1 Consortia of Prepared Slurries

Sample ID	Watermelon (%)	Tigernut	Total (%)
A	0.9L (30%)	2.1L (70%)	3L (100%)
B	2.1L (70%)	0.9L (30%)	3L (100%)
C	1.5L (50%)	1.5L (50%)	3L (100%)

### ➤ Determination of pH

An aliquot of the fermenting juice was collected daily for analysis. The pH values of the slurries were determined using a pH meter (model: PHS-3C), which was standardized with buffer 4, 7 and 9. Ten milliliters each of the samples were decanted into sterile beakers with distilled water used as a blank to set the meter to neutrality before taking the readings. The electrode was immersed into the beakers containing the slurries and left for 30secs at which the reading was constant (Ochai and Kolhatkar, 2008).

### ➤ Total Titratable Acidity

The total titratable acidity of the sample was determined as described by Ogu and Mgbebu (2011). Using phenolphthalein as indicator, 10cm<sup>3</sup> of the sample was measured into a conical flask and titrated against 0.1N solution of sodium hydroxide.

The total titratable acidity was calculated as:  $\frac{V_1 \times N \times 75 \times 100}{1000 \times V}$

Where

V<sub>1</sub> = Volume (cm<sup>3</sup>) of NaOH

V = Volume (cm<sup>3</sup>) of sample used

N = Normality of NaOH

### ➤ Proximate Analysis of the Fruit Blends

#### • Determination of Moisture Content

### ➤ Sample Preparation

Samples of tigernut and watermelon were purchased from Oja Oba market Ado-Ekiti, Ekiti State and transported in a sterile container to the laboratory for analysis. All the glassware utilized were sterilized in a hot air oven maintained at 160°C for one hour, while other materials were sterilized in an autoclave maintained at 121°C for 15 minutes. Healthy and matured tigernut tubers were selected. A total of 1.4 kg w/v of the chosen tigernuts were weighed out in sections, properly washed in two changes of clean water, and then steeped for twenty-four hours before usage. The soaked tigernuts (1.79kg) were blended with 1000 mL of distilled water using an industrial blender (Qasa model). The purchased watermelons were sliced using a sterile knife with the seeds and rind (exocarp) carefully removed from the pulp (mesocarp), 2.60 kg of watermelon was then blended using an industrial blender (Qasa model) with 500 mL of water used during the blending process. The resulting slurries were divided between the sterilized fermenters after being evenly mixed with a stirrer that has been cleaned. Three litres of the prepared slurries were dispensed into sterile containers labelled A, B, & C as shown below on Table 1.

Ten ml of the blended sample was measured in a clean crucible using sensitive balance. The crucible with the sample was placed in an air-dry oven at 105°C and left to stay overnight. Then crucible was transferred to oven again and weighted after 2 hours, this was repeated until constant weight was obtained. The moisture content (MC) may be calculated as this investigation was conducted twice. The difference in weight which corresponds to moisture content was recorded and percentage moisture content calculated (AOAC, 2019).

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

W<sub>1</sub> = weight of empty crucible

W<sub>2</sub> = weight of the crucible and the sample before drying

W<sub>3</sub> = Weight of the crucible with the dried sample (AOAC, 2019).

#### • Determination of Ash Content

The ash content was determined from the loss in weight that occurred during incineration of the evaporated sample at a temperature high enough to allow all organic matter to be burnt off without allowing appreciable decomposition of the ash constituents. Ashing was carried out in a muffle furnace subjected to heat at 550°C for 6 h (AOAC, 2016).

$$\text{Ash content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

$W_1$  = weight of empty crucible

$W_2$  = weight of the crucible and the sample before drying

$W_3$  = Weight of the crucible with the dried sample.

- **Fat Determination**

Clean and dried thimble were weighed ( $W_1$ ) and 5 g oven dried sample was added and re-weighed ( $W_2$ ). Round bottom flask was filled with petroleum ether (40-60°C) up to  $\frac{3}{4}$  of the flask. Soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boiled gently, the sample was put in the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 h. After the barrel of the extractor will be emptied, the condenser was removed and the thimble removed, taken into the oven at 100°C for 1 h and later cooled in the desiccator and weighed again ( $W_3$ ) (AOAC, 2019).

$$\% \text{ Fat} = \frac{W_2 - W_3 \times 100}{W_2 - W_1}$$

- **Crude Protein Determination**

One gram of the sample was introduced into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electro thermal heater until a clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50 mL and 5 of this was transferred into the distillation apparatus, 5 mL of 2% boric acid was added into a 100 mL capacity conical flask (the receiver flask) and four drops of methyl red indicator were added. 50% of NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Distillation was carried out in the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50 after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1 M HCl and the protein content calculated (AOAC, 2019).

$$\% \text{ protein} = \% N \times F$$

Where F = conversion factor 100 (% in food protein)

$$\text{Where } \% N = \frac{V_s V_b \times N \times 0.01401 \times 100}{W}$$

VS = vol. (mL) of acid required to titrate sample

VB = vol. (mL) of acid required to titrate blank

N = normality of acid (0.1)

W = weight of sample in grams

- **Determination of Total Carbohydrate**

The Total carbohydrate content of the sample was obtained as described by Moronkola *et al.* (2011), where the results from fat, protein, moisture and ash content analyses were added up and the carbohydrate content was calculated as follow:

$$\text{TC} = 100\% - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash}).$$

### III. RESULT AND DISCUSSION

- **Physicochemical Analysis and Proximate Value before Fermentation**

The physicochemical result and proximate composition of the fruit blends before fermentation is presented on Table 2 and Table 3 respectively. The pH varied between  $6.43 \pm 0.00$  and  $6.67 \pm 0.00$ . The blend of 70% watermelon and 30% tigernut had the lowest pH while the blend of equal volume had the highest pH. The Total Titratable Acidity varied between  $0.34 \pm 0.02^c$  g/L to  $0.49 \pm 0.24^a$  g/L. The blend of 70% watermelon and 30% tigernut had the lowest TTA value while the blend of 30% watermelon and 70% tigernut had the highest value. The moisture content varied between  $81.19 \pm 0.66^c$  % and  $92.55 \pm 0.52^c$  %. The blend of equal volume of the fruits had the lowest moisture content while the blend of 70% watermelon and 30% tigernut had the highest. The fat content varied between  $1.30 \pm 0.10^b$  % and  $2.17 \pm 0.10^a$  %. The blend of 70% watermelon and 30% Tigernut had the lowest value while the blend of equal volume had the highest. The ash content of the fruit blends varied between  $0.36 \pm 0.35^b$  % and  $0.80 \pm 0.16^a$  %. The blend of 70% watermelon and 30% tigernut had the lowest value while the blend of equal volume had the highest. The carbohydrate content varied between  $4.16 \pm 0.54^c$  % and  $13.67 \pm 0.72^a$  %. The blends of 70% watermelon and 30% tigernut had the lowest carbohydrate content while the blend of equal volume had the highest. The protein content of the blends varied between  $1.64 \pm 0.11^c$  % and  $2.21 \pm 0.03^a$  %. The blend 30% watermelon and 70% tigernut had the lowest protein content while the blend of 70% watermelon and 30% tigernut had the highest. The total sugar content of the fruit blends varied between 59.56 mg/g to 93.59mg/g. The blend of 30% watermelon and 70% tigernut had the lowest value while the blend of 70% watermelon and 30% tigernut had the highest.

Table 2 Proximate Composition Properties of Samples before Fermentation

Fruit blends	Moisture content (%)	Ash content (%)	Crude fat (%)	Crude protein (%)	Carbohydrate (%)
A	$88.48 \pm 0.04^d$	$0.70 \pm 0.03^a$	$1.53 \pm 0.14^b$	$2.21 \pm 0.03^a$	$7.09 \pm 0.12^b$
B	$92.55 \pm 0.52^c$	$0.36 \pm 0.35^b$	$1.30 \pm 0.10^b$	$1.64 \pm 0.11^c$	$4.16 \pm 0.54^c$
C	$81.19 \pm 0.66^c$	$0.80 \pm 0.16^a$	$2.17 \pm 0.10^a$	$2.18 \pm 0.00^a$	$13.67 \pm 0.72^a$

- Results are expressed as mean  $\pm$  Standard deviation
- Data having different superscripts down the column are significantly different ( $p < 0.05$ )
- Data having same superscripts down the column are not significantly different at ( $p < 0.05$ )

➤ Keys

- A: 30% watermelon + 70% tigernut
- B: 70% watermelon + 30% tigernut
- C: 50% watermelon + 50% tigernut

Table 3 Physico-Chemical Properties of Samples before Fermentation

Fruit blends	pH	TTA (g/L)
A	6.51 $\pm$ 0.00	0.49 $\pm$ 0.02
B	6.43 $\pm$ 0.00	0.34 $\pm$ 0.02
C	6.67 $\pm$ 0.00	0.40 $\pm$ 0.07

➤ Keys

- A: 30% watermelon + 70% tigernut
- B: 70% watermelon + 30% tigernut
- C: 50% watermelon + 50% tigernut

➤ Physicochemical Analysis and Proximate Value after Fermentation

The pH and TTA of the blends are presented on Figure 1. The pH varied between 3.15 to 3.16. The blend of 30% watermelon and 70% tigernut had the lowest pH while the blend of equal volume had the highest. The Total Titrable Acidity varied between 0.96% and 1.52%. The blend of 30% watermelon and 70% tigernut had the lowest value while the blend of 70% watermelon and 30% tigernut had the highest.

The proximate composition of the fermented fruit blends is shown on Figure 2. The moisture content of the fermented fruit blends varied between 97.02% and 98.57%. The blend of equal volume of the fruits had the highest moisture content while the blend of 30% watermelon and

70% tigernut had the lowest. The fat content of the fermented blends varied from 0.2% to 0.46%. The blend of 70% watermelon and 30% tigernut had the lowest fat content while the blend of 30% watermelon and 70% tigernut had the highest. The Ash content of the fermented blend varied between 0.37% and 0.48%. The blend of 70% watermelon and 30% tigernut had the lowest ash content while the blend of 30% watermelon and 70% tigernut had the highest. The carbohydrate content of the fermented blend varied between 0.00 and 0.74%. The blend of 70% watermelon and 30% tigernut as well as equal volume of the blend had the lowest carbohydrate content while the blend of 30% watermelon and 70% tigernut had the highest.

The protein content varied between 1.31% and 2.06%. The blend of 30% watermelon and 70% tigernut had the lowest protein content while the blend of equal volume had the highest. The sugar content of the fermented fruit blend varied between 0.55mg/g and 26.19mg/g. The blend of 50% watermelon and 50% tigernut had the lowest sugar content while the blend of 70% watermelon and 30% tigernut had the highest as shown on Figure 3.

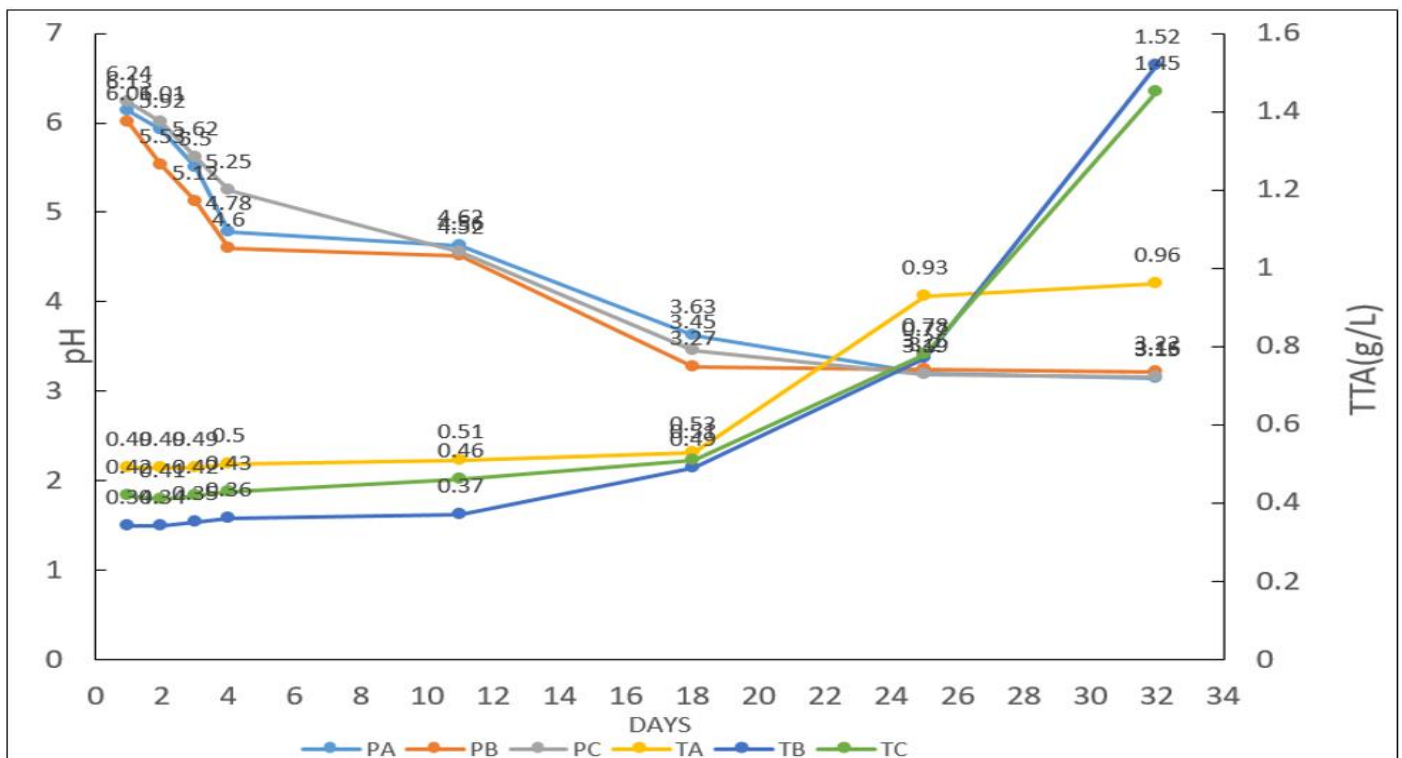


Fig 1 pH and Total Titrable Acidity in the Fermenting Fruit Blends.

➤ *Keys:*

- A: 30% watermelon + 70% tigernut
- B: 70% watermelon +30% tigernut,
- C: 50% watermelon + 50% tigernut,
- PA: pH of fruit blend

- PB: pH of fruit blend B
- PC: pH of fruit blend C,
- TA: Total Titrable Acid of fruit blend A
- TB: Total Titrable Acid of fruit blend B
- TC: Total Titrable Acid of fruit blend C

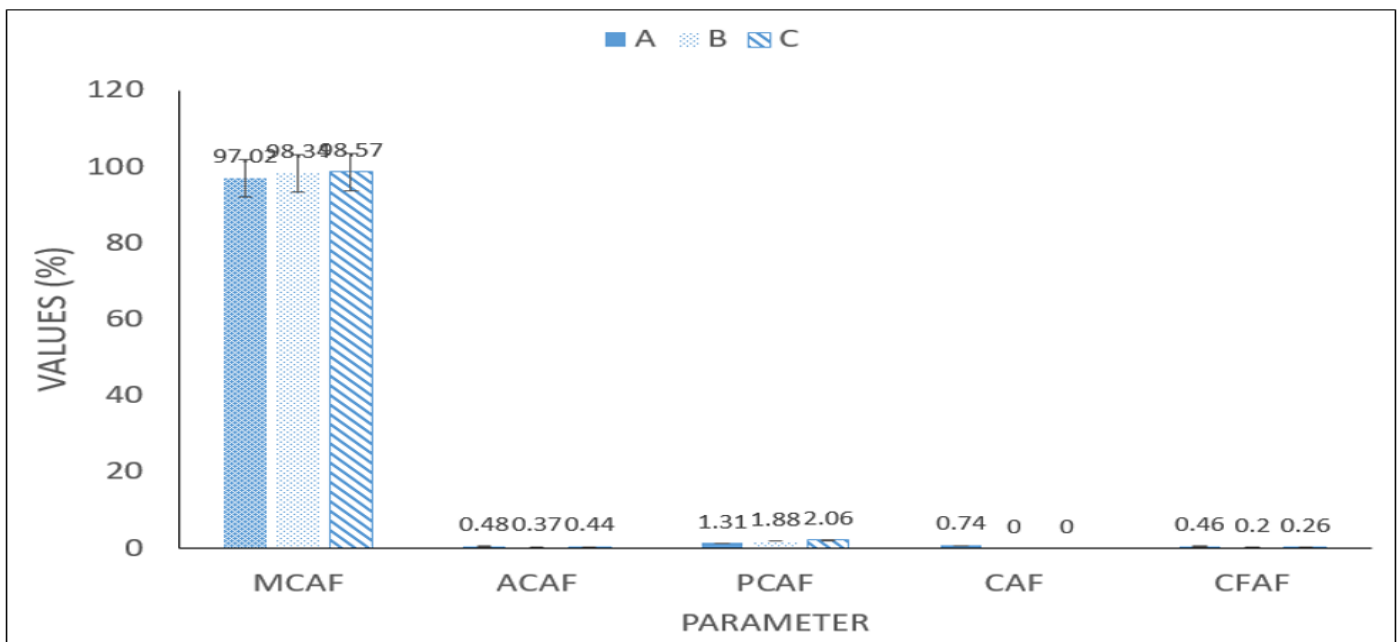


Fig 2 Proximate Composition of the Fruit Blends after Fermentation

➤ *Keys:*

- A: 30% watermelon + 70% tigernut,
- B: 70% watermelon +30% tigernut,
- C: 50% watermelon + 50% tigernut,
- ACAF: Ash content after fermentation,
- CAF: Carbohydrate after fermentation,
- CFAF: Crude Fat after fermentation,
- MCAF:Moisture content after fermentation,
- PCAF: protein content after fermentation

competitive advantage in natural fermentations (Chilaka et al., 2010).

Total Titrable Acidity (TTA) also increased throughout the fermentation period. This finding differs from the value (0.88%) reported by Ogodo et al. (2015) during the fermentation of watermelon, pawpaw and banana, (0.02 - 0.05%) recorded by Yabaya et al. (2016) during the fermentation of grape juice which were slightly lower. Although it is similar to (0.97%) reported by Ohoke et al. (2017) in the fermentation of tigernuts and well below (2.23%, 2.16% and 2.08%) reported by Kantiyok et al. (2021) in the fermentation of pawpaw and watermelon. The decrease in pH and increase in TTA may be due to the activities of the microorganisms which resulted in the production of organic acids from available sugar and nutrients present throughout the fermentation period for their growth and metabolism (Agbaje et al., 2015).

#### IV. DISCUSSION

Findings from this study showed that blends of watermelon and tigernut possess some attributes which can aid the transformation of this blends into other substrates such as low crude fibre, low protein content, low fat content and moderate Titrable Acid in the fermenting substrate after four weeks of fermentation process.

A reduction in the pH of the fermenting blends was observed after fermentation. This finding corroborates similar observations reported in the fermentation of sweet potato by Ray et al. (2011), sapota fruit (Panda et al., 2014) and fermentation of watermelon, pawpaw and banana (Ogodo et al., 2015). Fleet (2013) posited that, the decrease in the pH value may be due to the presence of lactic acid bacteria responsible for increased acidity during the fermentation of sugar substrate due to their proliferation in the substrates. Studies have also shown that during fermentation, low pH is known to give fermenting yeasts a

The proximate analysis result of the blend of fruits showed that, there was an increase in the moisture content of the three fruit blends, this corroborates those of Kantiyok et al. (2021) and Okeke et al. (2015) who both reported high moisture content during the fermentation of consortium of pawpaw and watermelon, pineapple and watermelon respectively. The addition of water to the mix(es) before the fermentation process could have resulted in the increase in moisture content (Ojokoh and Ojokoh, 2015).

The ash content reduced in fruit blend A and C after fermentation, but it increased in fruit blend B, this finding corroborates reports of Agbaje et al. (2015) who reported a

decrease in the ash level of fermented tigernut, although the values obtained in this present study were lower than 0.8% - 2.5% reported by Agbaje et al. (2015). Ash content is indicative of the minerals in a food sample; an increase in it during microbial fermentation may be due to incomplete mineral use by fermenting organisms throughout their metabolism, whereas a reduction indicates complete mineral utilization in the food sample (Ojokoh and Ojokoh, 2015).

Crude fat present in the fruit juices reduced after fermentation as the values obtained were lower than the values reported by Kantiyok et al. (2021) who observed a crude fat content of 7.96, 8.30 and 7.45% during fermentation of watermelon, pawpaw and a consortia of water melon and pawpaw respectively. This modest lipid change in the fermented product indicates that these substrates may be able to guard against excessive body lipids, and it also highlights the fermented product's beneficial nutritional qualities (Awe et al., 2013).

There was a decrease in the carbohydrate level of the final product, the values obtained were lower than the values observed by Kantiyok et al. (2021) during the fermentation of watermelon and pawpaw. The decrease in carbohydrate level observed closely agrees with the findings of Awe and Nnadoze (2015) who posited that, the decrease in carbohydrate content may be due to loss in sugar content resulting from the yeast cells' efficient breakdown and use of the sugar present in the substrate, thereby, causing it to ferment.

A decrease in the protein content of the fruit blends was also observed, this corroborates with the reports of Kantiyok et al. (2021) who observed that there was a decrease in the protein content of pawpaw and watermelon after fermentation. The protein content observed in this study was lower than the value (3.83%) reported by Kantiyok et al. (2021) in the fermentation of pawpaw and watermelon, it was however higher than the value (0.58%) reported by Yabaya et al. (2016) in the fermentation of grape. The increased change in sample B may be attributed to the opinion that microorganisms responsible for fermentation may have secreted extracellular enzymes which increased the protein content (Anyika, 2006; Agbaje et al., 2015) while the decrease in protein content after fermentation is good for maintenance of cellular organization of body system (Awe et al., 2013).

## V. CONCLUSION

Considering the determined quality parameters gotten from the natural fermentation of this blend of tiger nut and water melon, it was found out that these fermented juice contain low fat content which could provide protection against excess body lipids. The protein level and carbohydrate content of the fruit blends were also found to be comparable with established studies on the fermentation of other fruits.

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