

## Nutritional, Antioxidant and Quality Acceptability of Smoothies Supplemented with *Moringa Oleifera* Leaves

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### Abstract

This study evaluated the impact of the level of supplementation of *Moringa oleifera* leaves on the nutritional (proximate, mineral and vitamin content) as well as on the antioxidant capacities of smoothies made from a blend of pineapple, banana and apple. The beverage (smoothies) were supplemented with *Moringa oleifera* leaves at 1.5, 3.0 and 4.5% levels and evaluated for proximate, antioxidant (ferric reducing antioxidant power, FRAP and 2,2-Diphenyl-1-picrylhydrazyl, DPPH), mineral, vitamin, physicochemical and quality acceptability. The results showed that the moisture content of the beverage ranged between 49.24 – 78.62%, total ash: 1.01 – 9.71, crude fiber: 5.14 – 9.39%, crude fat: 0.72 – 1.86%, crude protein: 5.47 – 19.37% and carbohydrate: 3.65 – 16.99%. Calcium (12.03 -15.53) and potassium (17.22 -25.38) were the predominant mineral elements when compared to magnesium (1.51 – 3.05) mg/L. The vitamin contents ranged between 2.5 – 10.8 and 0.15 – 0.93 mg/L for vitamin C and E, respectively. Total phenolic contents ranged between 4.68 – 6.18 mg/ml while the total flavonoid contents ranged between 0.01 – 0.14 mg/ml. The radical scavenging abilities (DPPH) of the samples ranged between 16.05 – 88.77% while the ferric reducing antioxidant power (FRAP) ranged between 0.38 – 7.36 mg/ml. The brix values showed high sugar contents (15 – 18%) while the pH results showed that the sample was almost neutral at a range between 6.3 and 6.5. The overall quality (sensory) acceptability of the different quality parameters evaluated indicated that the control sample was more preferred. While supplementation with *Moringa oleifera* leaves significantly affected the sensory parameters, its addition nevertheless offers a potential avenue to obtain additional nutrients besides its improved antioxidative properties.

**Keywords:** chemical composition; smoothies; fruit blends; antioxidant properties; *Moringa oleifera* leaf; sensory evaluation

### 1. Introduction

While the awareness for consumption of fresh fruit or fresh fruit juices is globally acknowledged due to its health promoting abilities [1,2], an often over-looked aspect is its somewhat restricted nutritional composition. In addition, markets for smoothies-like product are already established in some developed countries generating over 2 billion USD and 141 million pounds in the USA and Great Britain, respectively [3]. Fruits are general rich sources of the micronutrients – minerals and vitamins [1]. Fruits are composed of varied micronutrients and a very viable and nutritionally efficient avenue of benefitting from the arrays of nutrients in the fruits is through the beverage called smoothies. Smoothies have been defined as a semi-liquid, smooth consistency mainly fruit-based products or beverages that are produced from a blend of different fruits in appropriate proportions [3]. Smoothies therefore being a blend of variety of fruits provides a better medium of obtaining the different micro-nutrients in the fruits. However, since fruits are generally low in protein content, the resulting beverages (smoothies)

will be deficient in this vital nutrient. A feasible means to address this is to supplement smoothies with other food materials including vegetable with high protein content such as *Moringa oleifera* leaves. Besides the need to encourage consumption of more fruits and vegetable, the concept of “smoothies also resulted from the need for consumers to enrich their diets [3]. Therefore, addition of *Moringa* leaf, reportedly containing high protein content into smoothies may provide another avenue to obtain additional benefits nutritionally and otherwise such as antioxidative. Therefore, the aims of this study were to evaluate the effects of *Moringa oleifera* leaves supplementation on the nutritional composition as well as the antioxidative properties of the smoothies.

## 2. Materials and Methods

### 2.1 Materials

Fresh ripe fruits – pineapple (*Ananas comosus*), banana (*Musa sapientum*) and apple (*Malus domestica*) and the vegetable (*Moringa oleifera* leaves) were bought from a local market in Akure, Ondo State, Nigeria. All the reagents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Method

The raw materials (fruits and vegetable) were sorted and thoroughly washed in clean water. Pineapples and bananas were peeled and the apples were cut open and the seeds removed. The cut fruits and vegetables were weighed using the blending ratio shown in Table 1 on an electronic weighing balance. One hundred milliliters (100 ml) of clean water was added to each sample using the measuring cylinder and blended with electric blender to obtain a semi-viscous liquid (smoothies).

**Table 1: Blending Ratio (g)**

	Pineapple (%)	Banana (%)	Apple (%)	<i>Moringa</i> leaf (%)	Total (%)
CS	45	40	15	-	100
TA	44.5	39.5	14.5	1.5	100
TB	44.0	39.0	14.0	3.0	100
TC	43.5	38.5	13.5	4.5	100

### 2.3. Analysis of the smoothies

The proximate composition (ash, fat, moisture content, protein and carbohydrate) and other analyses such as percentage brix, pH, titratable acidity, specific gravity of the product were carried out using AOAC (2012)

### 2.4. Determination of vitamin C content

The vitamin C content was determined using the ascorbic acid as the reference compound. Two hundred milliliter (200 ml) of the extract was mixed with 300 ml of 13.3% of TCA and 75 ml of DNPH. The mixture was incubated at 37°C for 3 hr and 500 ml of H<sub>2</sub>SO<sub>4</sub> was added and the absorbance was read at 520 nm [4]

### 2.5. Determination of mineral content

The ash obtained after ashing was washed into a volumetric flask and made up to 100 ml with 2% HNO<sub>3</sub>. It was analyzed using Buck scientific VGP 210 Atomic Absorption spectrophotometer.

### 2.6. Determination of total flavonoid content

The total flavonoid content of the extract was determined using a colourimeter assay developed by Bao *et al.* (2005) with some modifications. An aliquot (0.2 ml) of the extract was added to 0.3 ml of 5% NaNO<sub>2</sub> and after 5min, 0.6 ml of 10% AlCl<sub>3</sub> was added followed by the addition of 2 ml of 1 M NaOH after 6min and 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

### 2.7. Determination of total phenolic content

The total phenolic content of the extract was determined by the method of Nabavi, Ebrahinzadeh, Nabavi, & Jafari (2008) with some modifications. Two hundred microlitres (200 µl) of the extract was mix with 2.5 ml of 10% Folin Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture was subsequently incubated at 45 °C for 40 mins and the absorbance was measure at 700 nm. Garlic acid was used as standard phenol.

### 2.8. Determination of radical scavenging ability (DPPH)

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picrylhydrazyl) was carried out using the method of Nabavi *et al.* (2008) with slight modification. One milliliter (1 ml) of the extract was mixed with 1ml of the 0.4 mM methanolic solution of the DPPH the mixture was left in the dark for 30min before measuring the absorbance at 517 nm. The control consisted of methanol instead of the sample and the radical scavenging ability of the sample was calculated as:

$$\% DPPH = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

### 2.9. Determination of ferric reducing antioxidant power (FRAP)

The reducing property of the extract was determined by taking 250 ul of sample into test tubes (with distilled water as blank), 250 ul of 0.02M of phosphate buffer (pH 6.9) was added with 250 ul of 1% KFeCN. It was incubated for 20 mins at 50 °C thereafter, 250 ul of 10% TCA was added, 200ul of 0.1% freshly prepared of FeCl<sub>3</sub> (Ferric Chloride) was also added and 1 ml of distill water while the absorbance was read at 700nm

### 2.10. Sensory evaluation

The sensory evaluation of the samples of smoothie was carried out by 20 trained panelists comprising of students within the premises of the Federal University of Technology, Akure using a nine point hedonic scale where scores ranged from like extremely (1) to dislike extremely (9). Water was provided for each panelist for mouth rinsing after testing each product to avoid carry over effect.

### 2.11. Statistical analysis

Sample measurements were performed in triplicate and the data analyzed with SPSS version 17 while the means were separated for significant differences (p<0.05) using Duncan's Multiple Range test

## 3.0 Results and Discussion

### 3.1. Nutritional composition of smoothies supplemented with *Moringa oleifera* leaf

The effect of level of *Moringa oleifera* leaf supplementation on proximate composition of smoothies made from the blends of pineapple, banana and apple is shown in Table 2. As

expected, being beverages from fresh fruits, the smoothies showed high moisture content which significantly varied according to the level of *Moringa* leaf supplementation (49- 79%). The beverage may be able to serve as a thirst-quenching nonalcoholic beverage. The high moisture content may significantly affect the “shelf-stable” ability of the beverage except other preservative measures are taken. Total ash content is a reflection of the mineral composition of food samples. Addition of *Moringa* leaves significantly and progressively increased the total ash content of the smoothies. However, the significant increase in total ash did not result in substantial increase in the mineral element analyzed (Table 3). *Moringa oleifera* leaf which had been reported to contain **high protein content increased** the total crude protein content of the smoothies by 157, 217 and 254% for 1.5, 3.0 and 4.5 level of *Moringa oleifera* leaf supplementation, respectively. Efforts to improve nutritional composition of beverages, particularly with reference to protein content had been reported. One of such is the addition of yoghurt and or milk to smoothies [7,8]. The protein content of the control sample is relatively comparable to protein content in previous study but those of the supplemented samples showed significantly higher values ([9]. The fat content of the sample ranged from 0.72 % to 1.86% while the ash content ranged between 1.01 % and 9.71 %. The seemingly higher carbohydrate contents obtained for all the supplemented samples when compared to the control sample might be due to lower moisture contents in these samples rather than having more carbohydrate content than the control which obviously has considerably higher moisture content. The range of moisture and carbohydrate contents obtained in the current study are within those reported in a previous similar study [10].

**Table 2: Nutritional composition (%) of smoothies supplemented with *Moringa oleifera* leaves**

Samples	CS	TA	TB	TC
Moisture	78.62 <sup>a</sup> ±1.06	68.05 <sup>b</sup> ±1.06	53.31 <sup>c</sup> ±1.06	49.24 <sup>d</sup> ±1.06
Total ash	1.01 <sup>d</sup> ±0.07	5.62 <sup>c</sup> ±0.07	6.51 <sup>b</sup> ±0.07	9.71 <sup>a</sup> ±0.07
Crude fiber	9.39 <sup>a</sup> ±0.01	6.29 <sup>b</sup> ±0.01	5.14 <sup>da</sup> ±0.01	5.32 <sup>c</sup> ±0.01
Crude fat	1.86 <sup>a</sup> ±0.01	0.96 <sup>c</sup> ±0.01	0.72 <sup>d</sup> ±0.01	1.59 <sup>b</sup> ±0.01
Crude protein	5.47 <sup>d</sup> ±0.07	14.05 <sup>c</sup> ±0.07	17.33 <sup>b</sup> ±0.07	19.37 <sup>a</sup> ±0.07
CHO	3.65 <sup>d</sup> ±0.07	5.03 <sup>c</sup> ±0.07	16.99 <sup>a</sup> ±0.07	14.77 <sup>b</sup> ±0.07

Data presented as mean ±SD. Mean value in the same row with different superscript letters are significantly different (p≤0.05).

#### KEYS:

CS: 45% Pineapple, 40 % Banana, 15% Apple

TA: 44.5% Pineapple, 39.5% Banana, 14.5% Apple, 1.5% *Moringa* leaves

TB: 44% Pineapple, 39% Banana, 14% Apple, 3% *Moringa* leaves

TC: 43.5% Pineapple, 38.5% Banana, 13.5% Apple, 4.5% *Moringa* leaves

### 3.2 Mineral elements and vitamin compositions of smoothies supplemented with *Moringa oleifera* leaves

Part of the essential nutrients for proper growth and normal maintenance of the body are the micro nutrients. While some are required at more concentration – macro minerals (Na, K, P Ca) others are only needed at very small quantity – trace elements (e.g. Fe, Zn, Cu etc). These micro-nutrients play essential roles as co-factors for many metabolic processes in the body, hence their deficiency may result in significant nutritional deficiency diseases [11]. Table 3 shows the mineral element and vitamin composition of the smoothies. The content of calcium, potassium, magnesium and iron in the smoothies were determined. Potassium had the highest value and was highest in sample TC (25.38 mg/L) than the remaining formulated samples

(22.60 mg/L, 20.54 mg/L and 17.22 mg/L) respectively. The calcium content in the sample was also high in sample TC (15.53 mg/L) then followed by sample TA (14.04 mg/L), CS (12.03 mg/L) and TB (11.21 mg/L). The vitamin contents results on the other hand showed that the smoothies blends were low in the vitamins especially Vit C when compared to Ca and K contents. While vit c ranged between 2.5 (TB) to 10.8 (TC), vit E content ranged between 0.15 (TA) to 0.93 mg/100 g (TC). Sample TC has the highest vitamin C content compared to other and this may be due to the presence of higher percentage of *Moringa* leaves in the sample. The values obtained in this study are lower than those reported for juices from other fruits [9]. The vitamin E content of the smoothies ranges from 0.15 mg/100 g to 0.93 mg/100g.

**Table 3: Mineral composition (mg/L) of smoothies supplemented with *Moringa* leaves.**

Samples	CS	TA	TB	TC
Ca	12.03±0.13 <sup>c</sup>	11.21±0.13 <sup>d</sup>	14.08±0.13 <sup>b</sup>	15.53±0.13 <sup>a</sup>
K	22.60±0.10 <sup>b</sup>	17.22±0.10 <sup>d</sup>	20.54±0.10 <sup>c</sup>	25.38±0.10 <sup>a</sup>
Mg	3.05±0.14 <sup>a</sup>	2.58±0.14 <sup>b</sup>	1.51±0.14 <sup>d</sup>	1.96±0.14 <sup>c</sup>
Fe	0.008±0.05 <sup>c</sup>	0.009±0.05 <sup>c</sup>	0.018±0.05 <sup>a</sup>	0.014 ±0.05 <sup>b</sup>
Vit C	3.3±0.02 <sup>b</sup>	3.0±0.01 <sup>c</sup>	2.5±0.00 <sup>d</sup>	10.8±0.04 <sup>a</sup>
Vit E	0.46±0.02 <sup>c</sup>	0.15±0.01 <sup>d</sup>	0.63±0.02 <sup>b</sup>	0.93±0.01 <sup>a</sup>

Data presented as mean ±SD. Mean value in the same row with different superscript letters are significantly different ( $p \leq 0.05$ ).

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### 3.3 The phytochemical composition of smoothies supplemented with *Moringa* leaves

The result of phytochemical compositions (total phenolic and total flavonoid contents) of smoothies made from pineapple, banana, apple and *Moringa* leaves is depicted in Figure 1 A&B. Generally, the different smoothie's samples showed higher content of phenolic contents when compared to the flavonoid contents. This trend was also observed in a previous study [10]. The effects of the *Moringa* leaves of the phytochemical composition is reflected in the increase in total phenolic contents with increase in addition of *Moringa* leaves. However, the fact that similar trend was not obtained for the flavonoid contents and considering the generally low values obtained for the total flavonoid content compared to the total phenolic content might suggest the presence of more phenolic compounds in the fruits and vegetable used. Polyphenolic compounds including phenolic and flavonoid compounds are parts of the bioactive components in food which posse health promoting properties such as anti-inflammatory, antidiabetes, antihypertensive which have been the focus of many research studies [1,12–14]



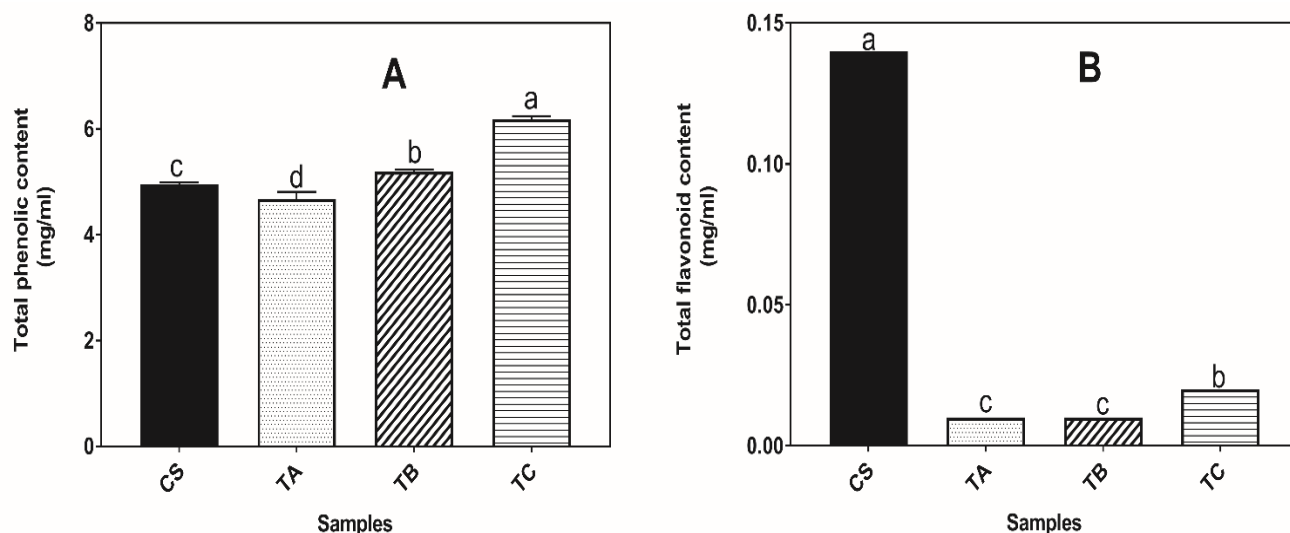


Figure 1: Percentage (mean  $\pm$  standard deviation, n=3) of total phenolic and total flavonoid contents of the smoothie (TPC and TFC). Bars with different letters have means values that are significantly different ( $p < 0.05$ )

#### KEY:

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#### 3.4. Antioxidant properties of smoothies supplemented with *Moringa* leaves.

Studies [2,15] have reported the roles of antioxidant in preventing or ameliorating the effects of free radical which have been implicated in many diseases including diabetes, hypertension, cancer etc. Phenols and flavonoid compounds are bioactive components of foods with health promoting properties including antioxidative and anti-inflammatory properties [16]. Diets (foods, including beverages) which is basic to human survival therefore provide an economically feasible and sustainable alternative avenue to prevent or modulate some of the challenges posed by free radicals compared to drugs which are often expensive besides their side effects. The antioxidant capacity of the smoothies is shown in Figure 2 A&B, the free radical scavenging property (DPPH) ranges from 16 % to 88.77 %. Both the free radical scavenging (DPPH) and ferric reducing abilities of the supplemented smoothies showed a similar trend with increase in their antioxidative potentials with increase in the level of *Moringa* leaf supplementation. That the two sample with higher *Moringa* leaf inclusion (TB and TC) which were not favorably accepted by the consumers in the sensory ratings (Table 4), may confirm the age-long saying that sweeter foods are not as beneficial health wise as the less sweet foods. In previous study [3], it was also observed that health promoting potentials of food was not directly correlated with the organoleptic properties as the sample with the best antioxidative properties was less acceptable in consumer evaluation.

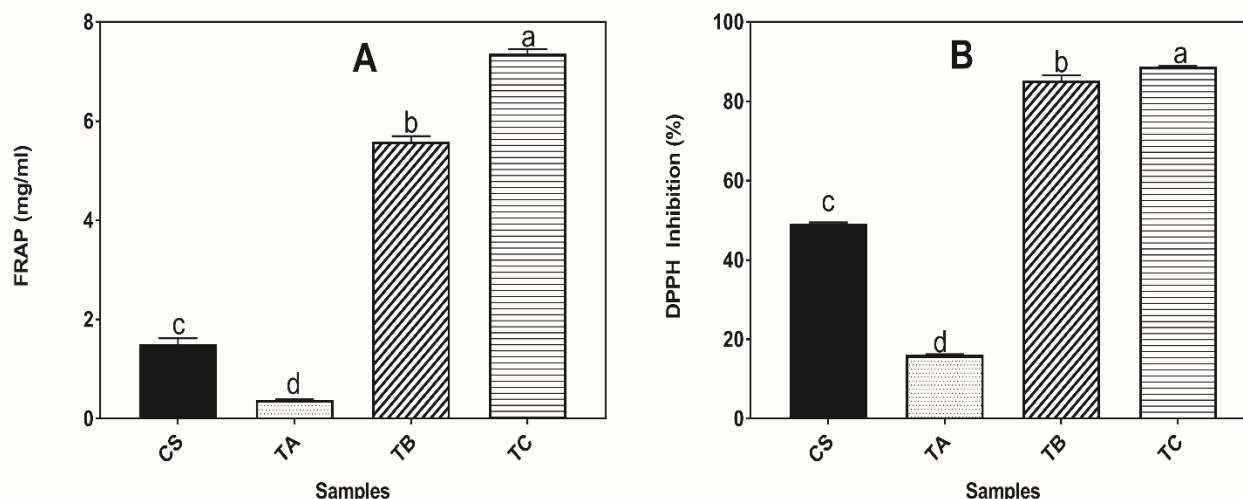


Figure 2: Percentage (mean  $\pm$  standard deviation, n=3) of antioxidant activities (FRAP and DPPH) of the smoothies. Bars with different letters have means values that are significantly different ( $p < 0.05$ ).

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#### 3.5 pH, TTA and percentage brix of smoothies supplemented with *Moringa* leaves

The physicochemical (pH, TTA and brix) properties of smoothies are shown in figure 3 A, B & C. The pH values revealed that all the samples, which were not significantly different tended more towards neutral pH and ranged between 6.3 and 6.51. These values are different from previous studies where the pH of fruit juices were mainly acidic [17,18]. The gradual decrease observed in the brix content of the samples is expected since brix measures the sugar contents of the foods. Obviously, *Moringa* leaves contains lesser content of sugar compared to other fruits hence the reduction in the brix values in *Moringa* supplemented smoothies. However, the current values are higher than those reported in literature [18]. The total titratable acid of the smoothies ranged from 0.0084 to 0.0098 and decreased with increased level of *Moringa* leaves supplementation. The low TTA values for the supplemented sample do not have corresponding effect in the taste raking by the consumer during sensory evaluation (Table 4). Though there is statistically significant differences in the TTA values, the range of the difference appeared to close to impact any significant effect on the taste parameter.

#### 3.6 Sensory evaluation of the smoothies supplanted with *Moringa* leaves.

Table 4 shows the result for the sensory characteristics of smoothie samples as compared to the control sample. There was a significant ( $p < 0.05$ ) difference between the control sample compared to other samples in term of taste, flavour, mouth feel, appearance and overall acceptability. It is evident that smoothies with 0% *Moringa* leaves (control sample) had the highest score (7.1) while smoothies from sample TA, TB and TC were fairly rated by panelists with respect to appearance. Appearance (colour) which results from the presence of the different color producing substances in the fruits and vegetable such as anthocyanins, carotenoids and chlorophylls is an important parameter used in judging the quality of foods especially fruit/vegetable based beverages like smoothies [1,19,20]. In terms of appearance, a

white/creamy appearance may be more appealing than greenish or other color impacted by the addition of other raw materials/ingredients. This however might be due to the effect of the *Moringa* leaves which impacted a greenish colour compared to the creamy appearance of the control sample. Similar trends were observed in the ratings of other parameters evaluated for the quality acceptability of the beverage. The apparently bitter taste of the leaf also significantly affected the taste as reflected with the level of *Moringa* leaves supplementation. The low ratings obtained for taste in *Moringa* leaf supplemented samples may be expected since the brix results which measures the sugar contents of the sample was low compared to the control especially for sample TC which was also rated lowest indicating reduced sweetness. Influence of materials incorporated into smoothies production on its consumers' acceptability have also been reported [3]. The indirect relationship between antioxidant properties and consumer acceptability of smoothies especial with the taste parameter have been reported [21]

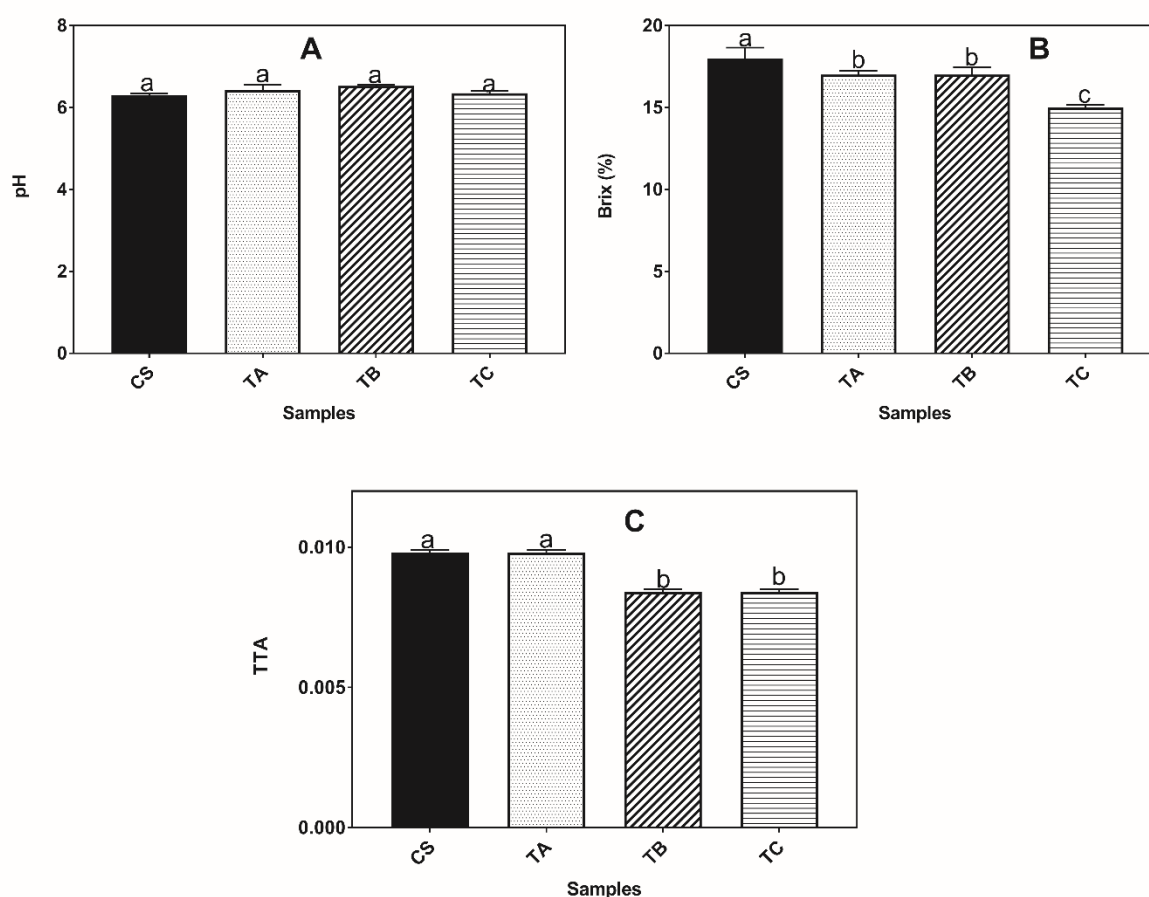


Figure 3: pH, Brix and total titratable acid (TTA) values (mean  $\pm$  standard deviation, n=3) of the smoothies. Bars with different letters have means values that are significantly different ( $p < 0.05$ )

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**Table 4: Sensory evaluation of the smoothies supplemented with *Moringa* leaves.**

Samples	Taste	Flavour	Mouth feel	Appearance	Overall acceptability	Mean
CS	6.5 ±0.4 <sup>a</sup>	6.5±0.2 <sup>a</sup>	6.2±0.3 <sup>a</sup>	7.1±0.2 <sup>a</sup>	6.7±0.3 <sup>a</sup>	6.6±0.3 <sup>a</sup>
TA	5.7 ±0.3 <sup>b</sup>	5.6±0.3 <sup>b</sup>	5.7±0.4 <sup>b</sup>	5.8±0.3 <sup>b</sup>	5.6±0.3 <sup>b</sup>	5.7±0.3 <sup>b</sup>
TB	4.9 ±0.3 <sup>c</sup>	5.1±0.4 <sup>c</sup>	4.5±0.3 <sup>c</sup>	4.8±0.3 <sup>c</sup>	4.9±0.3 <sup>c</sup>	4.8±0.3 <sup>c</sup>
TC	4.2 ±0.3 <sup>d</sup>	4.9±0.1 <sup>d</sup>	4.2±0.3 <sup>d</sup>	4.4±0.2 <sup>d</sup>	4.6±0.3 <sup>d</sup>	4.5±0.3 <sup>d</sup>

Values are means ± standard deviation of triplicate readings. Means with different alphabetical superscript in the same column are significantly different at (p<0.05)

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**CONCLUSION**

Supplementation of the fruit (pineapple, banana and apple) smoothies with *Moringa* leaves led to the production of highly nutritious beverages. The protein content of the smoothies was significantly improved with the addition of *Moringa* leaves. The antioxidative properties of the beverages were also significantly increased with the level of supplementation with *Moringa* leaves. Though inclusion of the leaves led to lower quality rating of the beverage specifically in terms of taste, flavour, mouthfeel, appearance and overall acceptability, Nevertheless, supplementation of smoothies with *Moringa* leaves may provide a better avenue to derive essential nutrients from the beverage apart from the improved antioxidative properties.

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