# THE IMPACTS OF LEMON JUICE ON THE PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF SMOOTHIES

**Taiwo Ayodele Aderinola**
Department of Food Science and Technology, The Federal University of Technology, PMB 704, Akure
+2347036569677
E-mail:taaderinola@futa.edu.ng

***Abstract***
*This study evaluated the natural preservative property and antioxidant effects of lemon on smoothies. The smoothies were prepared from blend of different ratio of fruits – A (40:30:30:0), B (39:29:29:3), C (38:28:28:6) and D (37:27:27:9) for pineapple, banana, apple and lemon respectively and stored at cool temperature (below 20 °C) for 48 h. The results showed that while FRAP (2.03 - 11.38 mg/g) and DPPH (4.67 - 5.32%) increased with increase in concentration of lemon, the reverse was observed for flavonoids (16.50 - 12.09%) and phenolic (0.15 – 0.13 mg/100g). Lemon addition also increased the vitamins C (15.93 - 55.36 mg/g) and A (477.72 - 1264.77 unit/g) contents. The samples were relatively acidic with pH range of 4.6 – 4.8 and titratable acidity range of 9.5 -19.2 g/100ml However, the results of sensory acceptability were not significantly affected by the different percentage of lemon used with respect to appearance, flavor, taste and overall acceptability of the smoothies produced from the fruit blends. Therefore, lemon juice up to the maximum 9% concentration used in this study could be used as a natural preservative for smoothies especially from the combination of pineapple, apple and banana.*

**Keywords:** smoothies; lemon; fruit smoothies; natural preservative, physicochemical

Received: 01.11.2018 Reviewed: 15.11.2018 Accepted: 28.11.2018

## 1. INTRODUCTION

Fruits and vegetables are essential in human nutrition and play vital roles in healthy living (Zacconi, Giosuè, Marudelli, & Scolari, 2015). In addition to the arrays of micronutrients (vitamins and minerals) they possess, they are also good sources of other phytochemicals with antioxidant properties (Lorente et al., 2014). However, reports abound that suggest that the daily intake of fruits and vegetable is below the recommended values of 400 (Naska et al., 2000). Apart from other reasons such as the access or cost of fruits and vegetables, the stress imposed on the palate may seriously constrain the amount consumed even where there is access. Eating healthily and keeping fit are essential in maintaining health (Nunes et al., 2016). While fruits and vegetables are currently being consumed or available mostly in processed forms – canned, concentrate, dried, paste and salad, smoothies provides another alternative to increase intake of fruits and vegetables. Besides being nutrient-dense as a result of the blending of two or more fruits/vegetable, it also reduces the stress involved in chewing the individual fruit or vegetable which invariably may reduce the quantity of such fruit/vegetable taken. Smoothies is a ready-to-drink beverage which provides a convenient way to blend varieties of foods – fruits, vegetables, nuts, milk(Castillejo, Martínez-hernández, Gómez, Artés, & Artés-hernández, 2015; Teleszko & Wojdyło, 2014). Studies have confirmed that regular consumption of fruits and vegetables confer some health benefits on the consumers (Andrés, Villanueva, & Tenorio, 2016; Balaswamy et al., 2013; Zacconi et al., 2015). Such health benefits include antioxidative, anti-diabetese, antihypertensive, anti-inflammatory and anti-carcinogenic among other (Castillejo et al., 2015; Mitjavila et al., 2013). Therefore, consumption of smoothies may provide a better avenue for getting synergistic effects of the various phytochemicals responsible for the observed health benefits. Giving that fruits and vegetables are packed with various nutrients that are essential to the proper growth and development of the body and the apparently impossibility of consuming all at the same time, smoothies therefore provides a medium to combine the fruit together, which will help to derive, maximally, the various nutritional benefits. Smoothies keeps the body hydrated, provides a boost of highly available antioxidants and beverage of high nutritional value to the body. It is also a way to improve digestion due to its high fiber content (Naska et al., 2000; Woodside, Young, & McKinley, 2013). Having such a product in the market, the consumers can be assured of a source of natural food supplement which would even be more enjoyable compared to other beverages but for its relatively short shelf life (Castillejo et al., 2015). Lemon (*Citrus limon*) is a small fruit commonly used for its culinary and non-culinary purposes (Julia, 1987). Lemon fruit (*Citrus limon*) is packed with essential phytochemicals and nutrients including vitamin c and flavonoid (Lorente et al., 2014). Due to its low pH content, it is able to serve as an antimicrobial (preservative) agent in beverages. Therefore the objectives of this study were to evaluate the potential of lemon to serve as a natural preservative as well as its effects on the physicochemical, antioxidant and quality acceptability of smoothies.

## 2. MATERIALS AND METHODS

### *2.1 Materials*

The materials used in this study were banana (*Musa sapientum*), apple (*Malus domestica*), pineapple (*Ananas comosus*), lemon (*Citrus limon* ). These raw materials were obtained from Shoprite stores in Akure, Ondo state. All the reagents used were of analytical grade.

### *2.2. Method*

The raw materials were rinsed, peeled, sliced into smaller sizes and the seeds of the lemon were removed before use. The sliced fruit chunks were blended separately using a domestic electric blender. The blends were mixed in their formulated proportions (Table 1) to obtain the resulting beverage. All smoothies were prepared without the addition of sugar, citric acid or any other forms of additive. The smoothie was stored for 72 hours at 4 $^{o}$C and physiochemical analyses were carried out simultaneously between the intervals of 5–6 hours.

**ioana verpoortenTable 1: Blending Ratio**

| Sample Codes | Pineapple (%) | Banana (%) | Apple (%) | Lemon (%) |
|---|---|---|---|---|
| 299 | 40 | 30 | 30 | 0 |
| 327 | 39 | 29 | 29 | 3 |
| 374 | 38 | 28 | 28 | 6 |
| 471 | 37 | 27 | 27 | 9 |

### *2.3. Determination of pH*

The pH of the smoothies were measured at an ambient temperature (28 ± 2 °C) using a digital Thermo Orion 868 pH meter (Thermo Fisher Scientific, Inc., Massachusetts, USA), which was calibrated with buffers (pH 4.0 and 7.0). Fifty millilitres (50 ml) of the smoothies was transferred into a beaker and the pH was determined after the meter had been calibrated.

### *2.4. Determination of Total Titratable Acidity (TTA)*

Total acidity of the smoothies was determined by titration method. Ten percent (10%) concentration of the beverage was prepared by taking 10 ml smoothies and making up to 100 ml using distilled water in a volumetric flask. An aliquot (10 ml) was then titrated against 0.1 N NaOH (Standardized using standard Oxalic acid) using phenolphthalein (2 drops) as indicator. The result was expressed as citric acid equivalence

$$Total\ Titratable\ Acidity\ \left(\frac{g}{100}ml\right) = Ave\ titre\ value\ \times 0.007 \times wgt\ of\ citric\ acid\ used$$

### 2.5. ***Determination of the brix content***

The brix was determined with a hand held refractometer, two to three drops of the sample were dropped on the refractometer screen and the measurement was viewed, facing a light source on the screen.

### *2.6. Viscosity Determination*

Viscosity was analyzed using Rion-viscotester, model VA-04F. The sample was placed in the measuring cup of the viscometer, the rotor was suspended into the sample to rotate and the measurement was taken in decipascal-second at 30 °C following some minutes of spindle rotation

### *2.7. Determination of Specific Gravity*

Specific gravity of the smoothies was determined using a 10 ml specific gravity bottle (SGB). The bottle was filled with sample and capped. Excess liquid was wiped off by blotting paper. Specific gravity (SG) was calculated as follows:

$$Specific\ gravity\ (SG) = \frac{(weight\ of\ SGB\ with\ sample)}{Weight\ of\ SGB\ with\ distilled\ water}$$

### *2.8. Determination of vitamin C content*

Vitamin C was determined according to the 2, 6 – dichlorophenol titermetric method (*Association of Analytical Chemists International. Official Methods of Analysis of AOAC*, 2012). A 2 g of the sample was homogenized with acetic acid solution and extracted. Vitamin C standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in 100 ml volumetric flask with distilled water. The solution was filtered out and 10 ml of the clear filtrate added into a conical flask in which 2.5 ml acetone had been added. This was titrated with indophenol dye solution (2, 6 – dichlorophenol indophenol) for 15 seconds. The procedure was followed for the standard as well. Ascorbic acid was calculated as:

$$Ascorbic\ acid\ (\frac{mg}{g}) = \frac{(C \times V \times DF)}{WT}$$

Where:

C = mg ascorbic acid, V = volume of dye used for titration, DF = Dilution Factor, WT = weight of sample (g)

### *2.9. Determination of Vitamin A*

The AOAC method (*Association of Analytical Chemists International. Official Methods of Analysis of AOAC,* 2012) using the colorimeter was adopted. This measures the unstable colour at the absorbance of 620 nm that result from the reaction between vitamin A and $SbL_3$ (Antimony (III) Iodide). Pyrogallol was added to 2g sample prior to saponification with 200 ml alcoholic KOH. The saponification took place in water bath for 30 minutes. The solution was transferred to a separating funnel where water was added. The solution was extracted with 1 –2.5 ml of hexane. The extraction was washed with equal volume of water. The extract was filtered through filter paper containing 5 g anhydrous $Na_2SO_4$ into volumetric flask. The filter paper was rinsed with hexane and made up to volume. The hexane was evaporated from the solution and blank. About 1 ml chloroform and $SbL_3$ solution were added to the extract and blank. The reading of the solution and blank was taken from the colorimeter adjusted to zero absorbance or 100%. Vitamin A content was calculated using:

$$\boldsymbol{Vitamin\ A\ Content} = \frac{(\boldsymbol{A_{620} \times SL \times V})}{\boldsymbol{WT}}$$

Where

$A_{620}$ = absorbance at 620nm

SL = slope of standard curve (Vit.A conc.) ÷ $A_{620}$ reading

V=Final volume in colorimeter tube

Wt= Weight of sample

### *2.10. Determination of mineral content*

The ash obtained after ashing was washed into a volumetric flask and made up to 100 ml with 2% $HNO_3$.It was analyzed using Buck scientific VGP 210 Atomic Absorption spectrophotometer

### *2.11. Determination of total flavonoid content*

The total flavonoid content of the extract was determined using a colorimeter assay as previously described (Bao, Cai, Sun, Wang, & Corke, 2005) with some modifications. An aliquot (0.2 ml) of the extract was added to 0.3 ml of 5% $NaNO_2$ and after 5min, 0.6 ml of 10% $AlCl_3$ 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.

***2.12. Determination of total phenolic content***

The total phenolic content of the extract was determined using a previously described method (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 $^oC$ for 40 mins and the absorbance was measure at 700 nm. Garlic acid was used as standard phenol.

***2.13. Determination of radical scavenging ability (DPPH)***

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) was carried out using an earlier described method (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:

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

***2.14. 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 $^oC$ thereafter, 250 ul of 10% TCA was added, 200ul of 0.1% freshly prepared of $FeCl_3$ (Ferric Chloride) was also added and 1 ml of distill water while the absorbance was read at 700nm

***2.15. 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.

***2.16. 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. RESULTS AND DISCUSSION

***3.1 Physicochemical properties of the smoothies pH, total titratable acidity (TTA), brix, specific gravity (SG) and viscosity***

The physicochemical properties determined for the product are: pH, titratable acidity (TTA) brix, specific gravity and viscosity. Figure 1 # and ## show the pH and TTA trends of the smoothies for 3 days of storage. An increase in pH and decrease in titrable acid as storage progressed for the period of storage was observed. Low pH obtained in the study is expected due to the acidic nature of fruits. This is also supported by previous studies (Castillejo et al., 2015; Di Cagno et al., 2011; Zacconi et al., 2015). However, the slight increases in pH observed in storage may be due to the degradation of ascorbic acid as previously observed (Keenan, Rößle, Gormley, Butler, & Brunton, 2012). The initial high TTA obtained in this study might be due to the influence of lemon juice addition (Xu et al., 2008). Earlier studies had indicated the inversely correlated relationship between pH and TTA as observed in the present study (Castillejo et al., 2015; Wang et al., 2014). Depending on the fruit or vegetable type used, TTA values could be significantly different. Some earlier reported values include 0.5 - 0.7 g malic acid/100 ml (Keenan, Brunton, Gormley, & Butler, 2011; Nunes et al., 2016), 9.81 (Zacconi et al., 2015). The soluble solid (brix) of the samples (Table 2) showed higher contents of soluble solid contents than what had been reported for similar drinks in the literature (Castillejo et al., 2015; Zacconi et al., 2015). The high value obtained in this study might be attributed to the pineapple content of the smoothies due to its higher sugar content and also to the differences in fruit samples used compared to those used in the reported studies. The values which ranged from 15.00 to 16.10 does not show any significant difference during storage. Stability of the soluble solids was also observed by other researchers (Li, Wang, Wang, & Liao, 2015). The result also showed that there was no significant difference (p<0.05) among the samples. The specific gravity of the samples (Table 2) ranged from 1.08 to 0.87 with slight differences among the samples possibly due to increased percentage of lemon juice which is evident by the reduction in specific gravity as the percentage inclusion of lemon juice increased, The viscosity of the samples is shown in table 7, which ranged from 233.00 to 200, sample A, B, C shows no significant difference and sample C shows little difference in the samples as the storage progressed from 0 hour to 48 hours.

### 3.2 Mineral and vitamin content of the smoothies

Essential micronutrients for human nutrition and health are found in most plants (Borah, Baruah, Das, & Borah, 2009). Different plants are composed with varied levels of these nutrients and reports abound linking them to promotion or sustaining of good health.

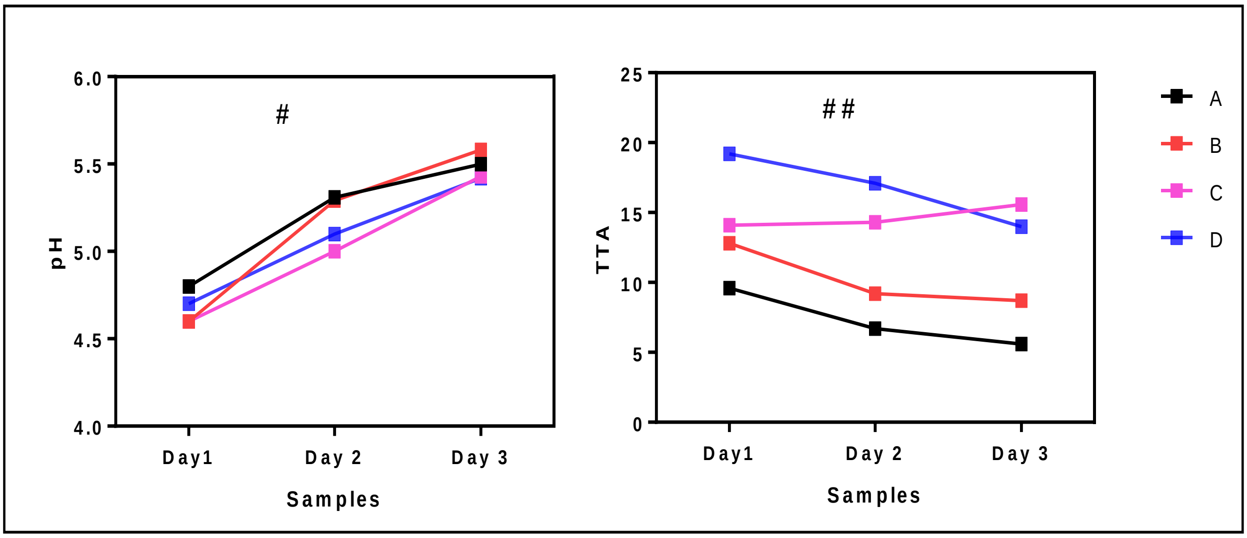


**Fig. 1: pH and titratable acidity of the smoothies**

KEY
A- (Control sample)-40% pineapple, 30%banana and 30% apple
B-39% pineapple, 29%banana, 29%apple and 3% lemon
C- 38%pineapple, 28%banana, 28%apple and 6% lemon
D-37% pineapple, 27% banana, 27% apple and 9% lemon

**Table 2: Brix, specific gravity and viscosity of the smoothies**

| | A | B | C | D |
|---|---|---|---|---|
| Brix | 16.00±0.00$^{a}$ | 15.00±0.00$^{a}$ | 16.00±0.00$^{a}$ | 16.00±0.00$^{a}$ |
| Specific gravity | 1.08±0.03$^{a}$ | 0.97±0.04$^{ab}$ | 0.96±0.05$^{ab}$ | 0.87±0.04$^{b}$ |
| Viscosity | 233.00±1.00$^{a}$ | 218.50±2.50$^{b}$ | 237.00±2.00$^{a}$ | 200.00±2.00$^{c}$ |

Means with different superscript along the same row are significantly different (p<0.05).

KEY
A- (Control sample)-40% pineapple, 30%banana and 30% apple
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D-37% pineapple, 27% banana, 27% apple and 9% lemon

Therefore, nutritional; information is important and is being used by public agencies and also by buyer who are getting more interested in nutritional benefits they stand to benefit from a particular food product (Borah et al., 2009). Mineral ions play vital roles in metabolism as transport co-factor and stabilize enzymes (Aberoumand & Deokule, 2009; Hood & Skaar, 2012). Table 3 shows the mineral contents of the samples. Major mineral present in the sample are potassium (91.40 - 99.50 ppm), sodium (62.25 - 69:00 ppm) and calcium (60.45 - 68.85 ppm). Studies on fruit smoothies had also reported higher content of potassium (Aderinola, 2018; Andrés, Tenorio, & Villanueva, 2014) Generally, there was more of the mineral elements in the control sample than any of the remaining samples. This may be due to higher contents of the fruits in the control sample compared to other samples. In a study on fruit jam, high content of potassium, sodium and calcium were also reported (Awolu, Okedele, & Ojewumi, 2018; Hanan & Rasha, 2012). Micro-minerals such as potassium, sodium, magnesium and calcium are important in proper functioning of the body. For instance, potassium serves as a co-factor in protein synthesis, enzyme activation, muscle contraction and regulation of blood pressure; calcium is essential for bone formation (Akinhanmi & Atasie, 2008; Awolu et al., 2018; Whitney & Rolfes, 1999). The result for the vitamin compositions of smoothies (Table 3) on the other hand was inversely correlated to the mineral element composition. While the control sample had higher mineral content in all the minerals tested for, samples with lemon juice were higher in vitamin contents and the more the percentage of lemon juice, the higher the vitamin contents. Apparently this was due to the addition of the lemon juice. The samples showed significantly higher content of vitamin A compared to vitamin c. However, the vitamin content obtained in this study is higher than the values previously obtained in a similar study (Aderinola, 2018). Vitamin A composition ranged from 477.72 to 1264.77 unit/g while vitamin C ranged from 15.93 to 55.36 (mg/g). Vitamin C is a water soluble vitamin, a major non-enzymatic antioxidant involved in radical scavenging in the body (Awolu et al., 2018).

**3.3 Phytochemical and antioxidant composition of the smoothies**

The result for the antioxidant composition of smoothies are shown in Figure 2 A&B with different levels of antioxidants in the samples. The ferric reducing antioxidant power (FRAP) of the samples (Figure 2A) ranged from 2.03 mg/g to 11.38 mg/g. Sample D which has the highest content of lemon (9%) shows the highest FRAP content of all the four samples.

**Table 3: Vitamin and Mineral Composition of Smoothies**

| Sample | A (control) | B (3%) | C (6%) | D (9%) |
|---|---|---|---|---|
| Vit A ( unit/g) | $477.72 \pm 0.27^{d}$ | $1109.71 \pm 0.13^{c}$ | $842.96 \pm 0.08^{b}$ | $1264.77 \pm 4.26^{a}$ |
| Vit C (mg/100 g) | $15.93 \pm 0.06^{d}$ | $54.77 \pm 0.11^{c}$ | $27.11 \pm 0.08^{b}$ | $55.36 \pm 0.08^{a}$ |
| Na (mg/L) | $69.00 \pm 0.20^{a}$ | $65.55 \pm 0.35^{b}$ | $62.25 \pm 0.15^{c}$ | $68.75 \pm 0.15^{a}$ |
| K (mg/L) | $99.50 \pm 0.50^{a}$ | $98.70 \pm 0.40^{a}$ | $91.40 \pm 0.20^{c}$ | $95.70 \pm 0.30^{b}$ |
| Ca (mg/L) | $68.85 \pm 0.25^{a}$ | $65.35 \pm 0.05^{b}$ | $61.65 \pm 0.15^{c}$ | $60.45 \pm 0.25^{d}$ |
| Mg (mg/L) | $5.92 \pm 0.03^{a}$ | $5.06 \pm 0.04^{c}$ | $5.54 \pm 0.05^{b}$ | $5.48 \pm 0.02^{b}$ |
| Fe (mg/L) | $1.60 \pm 0.03^{a}$ | $1.28 \pm 0.01^{b}$ | $1.20 \pm 0.0.1^{c}$ | $1.33 \pm 0.02^{b}$ |

Means with different superscript along the same row are significantly different (p<0.05).

KEY
A- (Control sample)-40% pineapple, 30%banana and 30% apple
B-39% pineapple, 29%banana, 29%apple and 3% lemon
C- 38%pineapple, 28%banana, 28%apple and 6% lemon
D-37% pineapple, 27% banana, 27% apple and 9% lemon

This might be as a result of the high content of vitamin C present in the sample (Fatin Najwa & Azrina, 2017). The DPPH radical scavenging capacity of the smoothies ranged from 4.67% to 5.32%. Sample D which has the highest content of lemon shows the highest DPPH content of all the four samples, This is due to high content of vitamin C present in the smoothies. The result for the phytochemical composition of smoothies (Figure 2 C&D) showed varied levels of the polyphenol properties of the samples. The flavonoids content of the samples ranged from 12.09% to 16.50%. Sample A which is the control sample has the highest flavonoids content of all the four samples. While the phenolic contents of the samples ranged from 0.15 mg/100g to 0.13 mg/100g.

### 3.4 Sensory evaluation and quality acceptability of the product

The sensory evaluation of samples is shown in Table 4. The control sample was slightly rated higher in taste compared to other samples which may be due to the absence of lime in the sample. Generally, there was no significant (p<0.05) difference in all the samples with respect to the appearance, flavor, taste, mouth feel and overall acceptability.

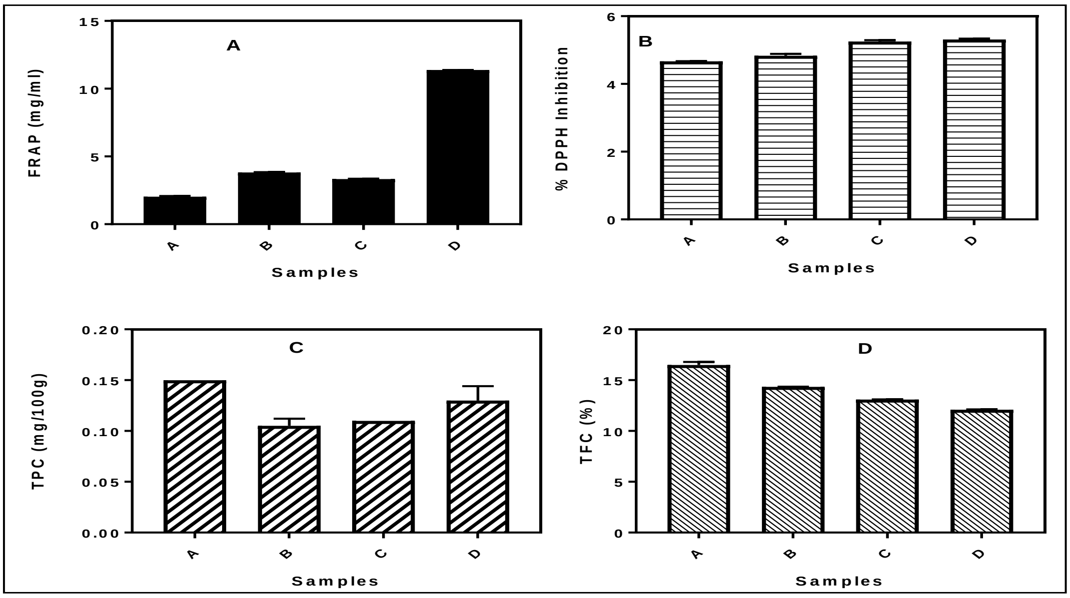


**Fig. 2. The antioxidant: Ferric reducing antioxidant power (A), DPPH radical scavenging ability; phytochemical: Total phenolic content (C) and Total flavonoid content (D) of the smoothies.**

**Table 4: Sensory Attributes of Smoothies**

| Sample | Appearance | Flavor | Taste | mouth feel | Overall acceptability |
|---|---|---|---|---|---|
| A (control) | 6.60±0.81[a] | 6.20±0.37[a] | 7.00±0.32[a] | 5.80±0.49[a] | 6.80±0.20[a] |
| B (3%) | 6.20±0.37[a] | 5.60±0.25[a] | 6.60±0.51[a] | 5.20±0.49[a] | 6.20±0.37[a] |
| C (6%) | 5.60±0.40[a] | 5.80±0.20[a] | 6.80±0.20[a] | 5.60±0.40[a] | 6.20±0.37[a] |
| D (9%) | 6.60±0.81[a] | 6.40±0.40[a] | 6.80±0.49[a] | 5.20±0.37[a] | 6.80±0.37[a] |

Means with different superscript within the same column are significantly different (p<0.05).

KEY
A- (Control sample)-40% pineapple, 30%banana and 30% apple
B-39% pineapple, 29%banana, 29%apple and 3% lemon
C- 38%pineapple, 28%banana, 28%apple and 6% lemon
D-37% pineapple, 27% banana, 27% apple and 9% lemon

## 4.CONCLUSION

Addition of lime juice as a natural preservative did not have any significant change in the overall acceptability of the smoothies for the 3 days of storage up to the maximum concentration (9%) used in this study.

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