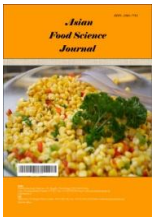

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Biochemical Characteristics and Antioxidant Properties of Citrus Juice from Lemon (*Citrus limon*), Lime *(Citrus aurantifolia)* and Grapefruit (*Citrus paradisi*) as Influenced by Degree of Ripening

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors worked on the research in a collaborative manner. Author MKB designed the study, wrote the protocol, performed the statistical analysis and proofread the draft of the manuscript. Author OOO carried out the literature searches, managed the analyses of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The influence of degree of ripening (unripe, half-ripe and ripe) of citrus fruits (lemon, lime and grapefruit) on the biochemical characteristics and antioxidant potential of their juices was investigated. The juice yield from the citrus fruits was affected by the level of ripening as the highest juice yield was obtained when the fruits were at ripe stage giving 25.2 mL/100 g (lemon), 43.3 mL/100 g (lime), and 21.1 mL/100 g (grapefruit). The biochemical characteristics of the citrus juices revealed that the pH values were generally increasing with an increasing level of ripening particularly for lime and grapefruit while the pH of lemon juice was decreasing with an increase in the degree of ripening. The lowest pH values exhibited by the citrus fruits were 2.87 (ripe lemon),

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2.16 (unripe lime), and 3.27 (unripe grapefruits). The total acidity of the citrus juices essentially exhibited an inverse relationship with their corresponding pH values. The highest ascorbic acid contents of the juices were 51.3 g/100mL (unripe lemon), 38.9 g/100mL (unripe lime), and 44.7 g/100mL (unripe grapefruit) while the highest total phenolic contents were 722 µg GAE/mL (unripe lemon), 207 µg GAE/mL (unripe lime), and 646 µg GAE/mL (unripe grapefruit); indicating a significant impact of degree of ripening on the parameters. The antioxidant activity of the citrus juices using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay revealed a significant influence of degree of ripening and juice volume on the activity. The percent inhibition of DPPH free radical by the juices was generally higher at an unripe stage of fruit maturity as well at greater juice volume. The ferric-reducing antioxidant potential (FRAP) of the citrus juices was also influenced by the level of ripening of the fruit. The FRAP values were generally higher at unripe stage which were 3.72 µ mol Fe(II)/g (unripe lemon), 4.98 µ mol Fe(II)/g (unripe lime) and 9.53 µmolFe(II)/g (unripe grapefruit).

Keywords: Fruit juice; lemon; lime; grapefruit; antioxidant; ripening.

1. INTRODUCTION

Citrus is one of the most important fruit crops in the world with global availability, varietal diversity and popularity contributing nutritional and health benefits to mankind [1,2]. Typical examples of citrus fruits with commercial relevance include orange (*Citrus sinensis*), tangerine (*Citrus tangerina*), lime (*Citrus aurantifolia*), lemon (*Citrus limon*), grapefruit (*Citrus paradisi*) and tangelo (*Citrus tangelo*), among others. Over the years, it has been observed that citrus fruits can offer nutritional and health benefits to consumers as citrus and citrus products are found to be a rich source of vitamins, minerals, dietary fibre and phytochemicals [3].

The utilization of citrus fruits is done in diverse ways including the production of fresh juice, squashes, citrus fruit powder, and marmalade [4]. The by-products from citrus fruits are also considered useful as some of them are used in livestock feed formulation, waste valorization, dietary fibre production, production of rind oil, pectin and organic acids [5-8]. The consumption of fresh citrus juices is particularly attractive to the consumers due to the perceived nutritional and health benefits derivable from the products. The benefits are usually associated, in part, with the presence of ascorbic acid which has been implicated to be involved in iron metabolism, the biosynthesis of carnitine, neurotransmitters, collagen and in the cross-linking of fibres in bones; and is also a co-factor in various enzymatic and hormonal processes [9,10]. The juices from citrus fruits particularly from lemon (*Citrus limon*) and lime (*Citrus aurantifolia*) have been in use in the indigenous system of medicine for the management of hypertension and other cardiovascular diseases, though the mechanism of action by which they exert their therapeutic action was not well understood [11].

The ripening process in fruits generally is an important phenomenon that is usually accompanied by biochemical and physiological changes normally driven by the coordinated expression of fruit-ripening-related genes [12]. In citrus fruits, the ripening process is mostly classified as non-climateric due to the absence of ripening-associated increase in respiration and in ethylene production [12]. Major changes in biochemical constituents of citrus fruits during ripening are associated with ascorbic acid, phenolic compounds, sugars, minerals, and other organic acids, among others [9].

Many researchers had worked on the effect of ripening on some of the biochemical constituents of citrus fruits. Fattahi et al. [13] assessed the role of ripening on some quality parameters of three citrus species while Rekha et al. [9] examined the influence of two stages of ripening on some constituents of citrus juices. Moulehi et al. [14] also investigated the impact of variety and ripening on the phenolic composition and antioxidant activity of only two types of citrus fruit while Bermejo and Cano [15] studied the effect of ripening on the nutritional constituents of twenty citrus cultivars.

Therefore, the present study was aimed at examining the influence of three different ripening stages (mature unripe, half-ripe, and ripe) on some biochemical constituents of juices from the selected citrus species (lemon, lime, and grapefruit) with a view to relating them to their antioxidant potentials.

2. MATERIALS AND METHODS

2.1 Sample Collection and Grouping

Fully matured green citrus fruits (lemon [*Citrus limon*], lime [*Citrus aurantifolia*] and grapefruit [*Citrus paradisi*]) were harvested by plucking manually from each tree within the Teaching and Research Farm, Federal University of Technology, Akure (FUTA), Nigeria; and kept at ambient temperature ($28^{o}\pm2^{o}$C) in separate perforated plastic containers respectively. They were individually allowed to ripen naturally from where they were grouped into three different ripening stages based on respective colour differential (unripe, half-ripe and ripe) using the International Standard Colour Chart [16].

2.2 Extraction of Juice from Selected Citrus Fruits

Each type of the selected citrus fruits, at different ripening stages, was washed thoroughly in distilled water. The juices were extracted by peeling and cutting the fruits into half and carefully squeezing manually to extract the juices, using a citrus squeezer. The collected juice was filtered using muslin cloth and the pulp-free juice was collected in clean stainless containers after which it was kept under freezing condition (0 ± 1^{o}C) until required.

2.3 Determination of Juice Yield

The juice yield from each type of the selected citrus fruits was measured in mL/g. The overall yield was calculated by using the following formula:

Juice yield= Juice extracted (mL) / Fruit weight (g) Eq(1)

2.4 Determination of pH and Total Acidity

The pH of fresh juices extracted was measured by using a digital pH meter (model WPA CD70, India) immediately after extraction. The pH meter was first standardized using buffer solution of pH of 4.0 and 7.0. The pH of each sample was then measured accordingly and after each determination, the pH probe was rinsed with distilled water and blotted dry [17].

Total acidity of the juices was determined by titration method as described by Rekha et al. [9]. Ten millilitres (10 mL) of each juice were measured into a volumetric flask and then made up to 100 mL mark using distilled water. Ten millilitres (10 mL) each of the diluted juices were titrated against 0.1N NaOH using Phenolphthalein indicator while the end-point was noted (the colour changed from colourless to pale pink). The total acidity (g/100 mL) was then calculated in terms of citric acid as follows:

Total acidity (g/100 mL) = mL of titrant x Normality of titrant x Equivalent weight of citric acid/ Sample juice volume Eq(2)

2.5 Evaluation of Ascorbic Acid Content of Juice Samples

The ascorbic acid content was determined using the method as described by Singleton et al. [18]. About 200 µl of each juice extract was pipetted and mixed with 300 µl of 13.3% of trichloroacetic acid (TCA) and 75 µl of dinitrophenylhydrazine (DNPH). The mixture was incubated at 37^{o}C for 3 h and thereafter 500 µl of 65% H_2SO_4 was added followed by reading of the absorbance at 520 nm using a spectrophotometer (Model SP9 Pye Unicam, UK). Standard solutions of ascorbic acid (L-ascorbic acid) were prepared and run using several concentrations between 10 and 80 g/100 mL (10, 20, 30, 40, 50, 60, 70 and 80 g/100 mL). A standard curve was then prepared by plotting the absorbance value against each concentration. The ascorbic acid value for each sample was then determined using this standard curve. Blank sample was treated similarly through the entire determination. The ascorbic acid content was measured as g/100 mL.

2.6 Evaluation of Total Phenolic Content of Juice Samples

The total phenolic content in each of the fruit juice samples was estimated by Folin-Ciocalteu method as described by Thimmaiah [19]. Half millilitre (0.5 mL) of each fruit juice was mixed with 2.5 mL of distilled water. To this, 0.5 mL of Folin-Ciocalteu reagent (1:1) was added and incubated for 3 minutes. To each tube, 2 mL of 20% sodium carbonate was added and the tubes were kept in boiling water bath for 1 minute. Tubes were cooled and the absorbance of reaction mixture was read at 650 nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/mL). Total phenolic content was estimated as µg Gallic acid equivalent (GAE)/mL of fruit juice.

2.7 Determination of Antioxidant Activity of Juice Samples Using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant activity of the fruit juice samples was determined as described by Kekuda et al. [20] on the basis of scavenging ability of the fruit juices on DPPH free radical. Different concentrations of juices were prepared (from 20 µL to 100 µL/mL) in methanol. In clean and labeled test tubes, 2 mL of DPPH solution (0.002% in methanol) was mixed with 2 mL of different concentrations of fruit juices separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using a spectrophotometer (Model SP9 Pye Unicam, UK). The absorbance of the DPPH (control) was also noted. The scavenging activity of the juices was calculated using the formula:

$$\text{Scavenging activity (\% Inhibition)} = [(A - B) / A] \times 100 \quad \text{Eq (3)}$$

where,

A= Absorbance of DPPH (control).
B=Absorbance of DPPH and fruit juice combination.

2.8 Determination of Antioxidant Activity of Juice Samples Using Ferric Reducing Antioxidant Potential (FRAP) Assay

The method as described by Kekuda et al. [20] was used to determine the antioxidant activity of juice samples using ferric reducing antioxidant potential (FRAP) assay. About 0.25 mL of the juice sample was mixed with 0.25 mL of 200 mM of sodium phosphate buffer pH 6.6 and 0.25 mL of 1% potassium ferrocynanide. The mixture was incubated at 50^{o}C for 20 min, thereafter 0.25 mL of 10% tricholoroacetic acid (TCA) was also added and centrifuged at 2000 rpm for 10 min. One millilitre (1 mL) of the supernatant was mixed with 1 mL of distilled water and 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 min. Standards of known Fe (II) concentrations ($FeSO_4.7H_2O$) were run in triplicate using several concentrations between 25 and 1000 µM (25, 50, 75, 100, 150, 200, 500 and 1000 µM). A standard curve was then prepared by plotting the average FRAP value for each standard versus its concentration. The FRAP values for the samples were then determined using this standard curve. The values were reported as µ mol Fe(II)/g.

2.9 Statistical Analysis

All determinations reported in this study were carried out in triplicates. In each case, a mean value and standard deviation were calculated. Analysis of variance (ANOVA) was also performed and separation of the mean values was by Duncan's Multiple Range Test at $P<0.05$; using Statistical Package for Social Scientists (SPSS) software, version 10.0, on a personal computer.

3. RESULTS AND DISCUSSION

3.1 Yield of Juice from Lemon, Lime and Grapefruit as Influenced by Degree of Ripening

The influence of degree of ripening on the yield of citrus juice from lemon, lime and grapefruit is presented in Table 1. The mature unripe lemon, lime and grapefruit generally had the lowest juice yield, when compared with half-ripe and ripe counterparts, ranging between 11.2 and 22.2 mL/100 g with significant differences at $P<0.05$. The lowest juice yield in the mature unripe fruit may be attributed to difficulty in juice removal from the fruits due to the seeming highest structural integrity which the fruits possessed at that stage. It had earlier been observed that at mature unripe stage of fruits, the softening of fruit structural integrity due to enzyme-mediated alterations in the composition of cell wall is not yet initiated [21]. The juice yield in the half-ripe lemon, lime and grapefruit was 23.1, 27.2 and 17.2 mL/100 g respectively while that of the ripe lemon, lime and grapefruit was 25.2, 43.3 and 21.1 mL/100 g respectively. It was generally observed that the juice yields were increasing with higher ripening level of the fruits. From Table 1, the juice yield from lemon fruit increased from the initial 22.2 mL/100g (mature unripe) to 25.2 mL/100 g (ripe) while the yield from lime fruit increased from 15.1 mL/100g (mature unripe) to 43.3 mL/100 g (ripe); all with significant differences at $P=0.05$. In the case of juice yield from grapefruit, it increased from the initial 11.2 mL/100g (mature unripe) to 21.1 mL/100 g (ripe) with significant difference at $P=0.05$. Previous

researches had observed that during fruit ripening, softening of the plant tissue could lead to the release of cell content thereby leading to higher juice yield [22]. Other factors that can influence juice yield of citrus fruit include fruit type and dry matter composition of the fruit [23]. In general, the citrus juice is naturally enclosed inside juice sacs which are usually numerous and attached compactly to each other in mature fruit [24]. Therefore, during ripening, the juice sacs normally become weakened due to the softness of the cell wall with concomitant decrease in the structural integrity and increase in intracellular spaces of the fruit [25]. All these occurrences facilitated an increase in the juice yield of half-ripe and ripe citrus fruit counterparts.

3.2 Selected Biochemical Characteristics of Citrus Juice from Lemon, Lime and Grapefruit as Influenced by the Degree of Ripening

Table 2 shows the effect of degree of ripening on selected biochemical characteristics of juice from lemon, lime and grapefruit. The pH of lime with respect to mature unripe, half-ripe and ripe was 2.16, 2.22 and 2.77 respectively while that of the grapefruit with respect to mature unripe, half-ripe and ripe was 3.27, 3.57 and 3.75 respectively. The pH trend as regards lime and grapefruit was that the values were increasing as the degree of ripening was increasing. However, in the case of lemon, the pH level for mature unripe, half-ripe and ripe was 3.28, 2.98 and 2.87 respectively; indicating a gradual decrease in pH level as the degree of ripening was increasing. The pH level in the citrus fruits as shown in this study has laid credence to the fact that during maturity, citrus pH may exhibit different responses depending on fruit types [26].

The total acidity of the citrus juice from lemon, lime and grapefruit Table 2 was observed to be indirectly related to the pH trend. The total acidity of lime juice with respect to mature unripe, half-ripe and ripe was 8.98, 8.81 and 8.56 g/100 mL respectively. This trend indicates that the total acidity was decreasing in both lime and grapefruit juices as the degree of ripening was increasing. In the case of lemon juice, the total acidity with respect to mature unripe, half-ripe and ripe was 4.14, 5.48 and 5.93 g/100 mL respectively; indicting a gradual increase in total acidity as the degree of ripening was increasing. This observation is in conformity with an earlier finding that the total acidity in lemon juice tended to increase with maturity thereby resulting in low pH level [27]. In general, earlier researchers had indicated that the organic acids in the citrus juices are constituted by citric, malic, and succinic acids though citric acid is the prevailing component [27,28]. It had also been observed that the decrease and utilization of organic acids during citrus fruit maturity usually lead to the synthesis of many flavour and aromatic compounds as metabolites [29].

The ascorbic acid content of citrus juice from lemon, lime and grapefruit Table 2 was observed to decrease as the degree of ripening was increasing. The ascorbic acid content of lemon juice with respect to mature unripe, half-ripe and ripe was 51.3, 32.4 and 29.2 g/100 mL respectively. In the case of lime juice, the ascorbic acid content with respect to mature unripe, half-ripe and ripe was 38.9, 26.1 and 21.8 g/100 mL respectively while grapefruit juice also exhibited ascorbic acid content of 44.7, 25.2 and 14.5 g/100 mL for mature unripe, half-ripe and ripe respectively. Apart from the degree of ripening, the variability in the ascorbic acid content of citrus fruit juices could also be influenced by other factors including fruit type, variety, climate, and handling, among others [30]. It had generally been observed that the significant contribution of citrus fruits to human nutrition and health has to do with the presence of ascorbic acid in them [31].

The total phenolic content of citrus juice from lemon, lime and grapefruit Table 2 was observed to decrease in value as the degree of ripening was increasing. The total phenolic content of lemon juice with respect to mature unripe, half-ripe and ripe was 722, 531 and 414 µg GAE/mL respectively. In the case of lime juice, the total phenolic content was 207, 141 and 99 µg GAE/mL for mature unripe, half-ripe and ripe and respectively. The grapefruit juice from mature unripe, half-ripe and ripe also exhibited the total phenolic content of 646, 338 and 291 µg GAE/mL respectively. The general gradual decrease in the concentration of total phenolic content in the juices as ripening progressed can be attributed to enzyme-mediated degradation of phenolic-related compounds such as bitter principles, flavanoids and tannins, among others [21]. In general, the phenolic compounds are regarded as secondary metabolites and are responsible for such functions as pigmentation and resistance to pathogens and predators, the roles attributed to their phytoalexin properties and potent astringency [32,33].

Table 1. Influence of degree of ripening on the yield of citrus juice from lemon, lime and grapefruit

Citrus type	Juice yield (ml/100 g)[1]		
	Mature unripe	Half-ripe	Ripe
Lemon (*Citrus limon*)	22.2±0.8^{aBC}	23.1±0.9^{bB}	25.2±0.6^{bA}
Lime (*Citrus aurantifolia*)	15.1±0.4^{bC}	27.2±0.5^{aB}	43.3±0.8^{aA}
Grapefruit (*Citrus paradisi*)	11.2±0.7^{cC}	17.2±0.4^{cB}	21.1±0.7^{cA}

[1]*Mean values within the same column having the same lowercase superscript are not significantly different at P=0.05; while the mean values within the same row having the same uppercase superscript are not significantly different at P=0.05*

Table 2. Effect of degree of ripening on selected biochemical characteristics of citrus juice from lemon, lime and grapefruit

Citrus type	Degree of ripening	Biochemical characteristics[1]			
		pH	Total acidity (g/100 mL)	Ascorbic acid (g/100 mL)	Total phenolic content (µg GAE/mL)
Lemon (*Citrus limon*)	Mature unripe	3.28±0.02^{c}	4.14±0.01^{f}	51.3±1.6^{a}	722±2.3^{a}
	Half-ripe	2.98±0.01^{d}	5.48±0.01^{e}	32.4±1.3^{d}	531±3.6^{c}
	Ripe	2.87±0.01^{e}	5.93±0.02^{d}	29.2±0.7^{e}	414±4.3^{d}
Lime (*Citrus aurantifolia*)	Mature unripe	2.16±0.01^{h}	8.98±0.04^{a}	38.9±1.8^{b}	207±2.7^{g}
	Half-ripe	2.22±0.01g	8.81±0.02^{b}	26.1±0.4^{f}	141±4.1^{h}
	Ripe	2.77±0.01^{f}	8.56±0.01^{c}	21.8±0.2^{g}	99±3.3^{i}
Grapefruit (*Citrus paradisi*)	Mature unripe	3.27±0.01^{c}	2.89±0.03^{g}	44.7±1.4^{c}	646±1.9^{b}
	Half-ripe	3.57±0.02^{b}	2.85±0.02^{g}	25.2±0.5^{f}	338±2.5^{e}
	Ripe	3.75±0.02^{a}	2.19±0.01^{h}	14.5±0.2^{h}	291±3.6^{f}

[1]*Mean values within the same column having the same superscript are not significantly different at P= 0.05*

3.3 Influence of Degree of Ripening and Juice Volume on the Antioxidant Activity of Juice from Lemon, Lime and Grapefruit Using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Free Radical Assay

Fig. 1. shows the effect of degree of ripening and juice volume on the antioxidant activity of lemon juice using DPPH free radical assay. The percent inhibition of DPPH free radical by lemon juice was generally highest with the unripe lemon followed by the half-ripe and ripe respectively. Similarly, at 20 µL juice volume, the percent inhibition of DPPH free radical was 3.8, 16.6 and 27.9% for ripe, half-ripe and unripe lemon juice respectively while at 100 µL juice volume, the percent inhibition was 28.8, 34.2 and 35.9% for ripe, half-ripe and unripe lemon juice respectively. These findings are essentially revealing that the percent inhibition of DPPH free radical by lemon juice was decreasing as the level of ripening was increasing. The implication of the findings therefore is that it is re-emphasizing the ability of lemon juice to inhibit DPPH free radical while such inhibition could be influenced by such factors as degree of fruit ripening and volume of the juice. The antioxidant ability of lemon juice is connected with the biochemical constituents it contains such as ascorbic acid and phenolic compounds Table 2. The higher the concentration of these compounds in the juice, the higher the free radical inhibition potential. Niki et al. [34] had observed that ascorbic acid is particularly regarded as a dietary antioxidant while the presence of phenolic compounds and ascorbic acid in diet could individually function as scavenging antioxidants whereby they remove reactive oxygen species (ROS) rapidly before such species attack biologically essential molecules [35,36].

Fig. 2 shows the effect of degree of ripening and juice volume on the antioxidant activity of lime juice using DPPH free radical assay. The percent inhibition of the free radical by lime juice was generally observed to decrease as the degree of ripening was increasing. The value-ranges for the percent inhibition of the free radical by lime juice, at different juice volumes, were 24.4–34.8% (unripe lime), 21.9– 32.3% (half-ripe lime), and 11.4–29.6% (ripe lime). The implication of these findings is that lime juice also exhibited antioxidant capability and it essentially displayed a dose-dependence activity as its free radical scavenging capability (inhibition of free radical) was dependent on its concentration or volume [11].

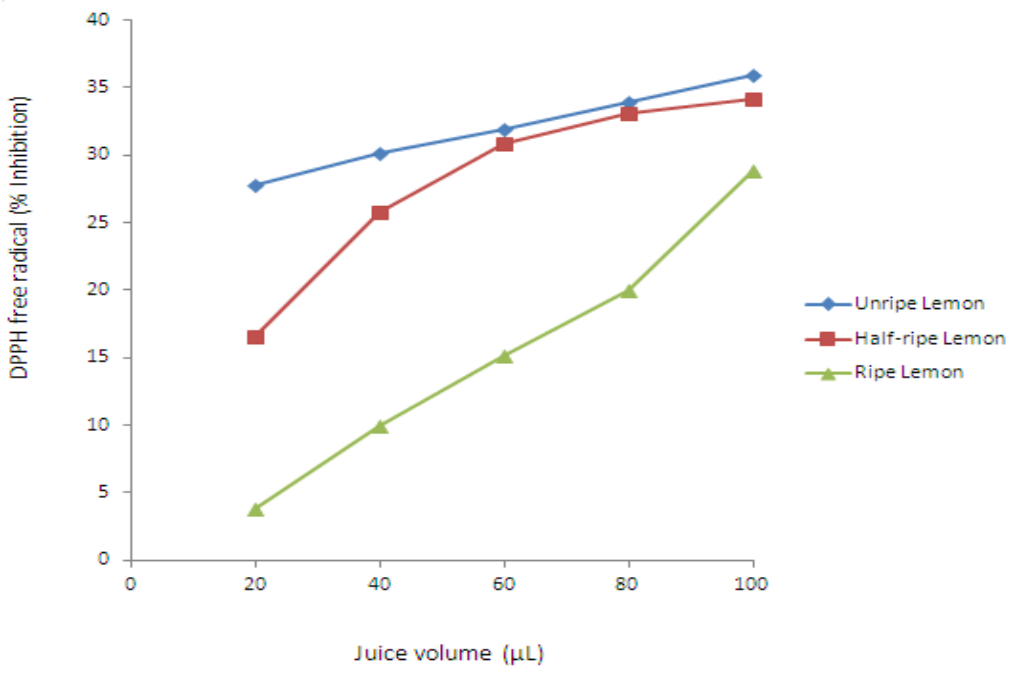

Fig. 1. Effect of degree of ripening and juice volume on the antioxidant activity of lemon juice using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay

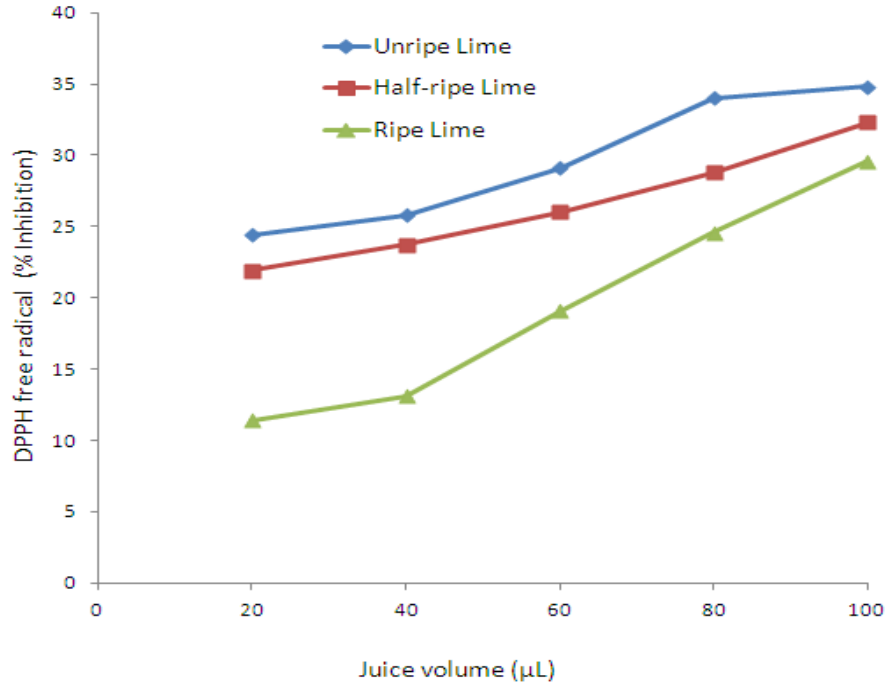

Fig. 2. Effect of degree of ripening and juice volume on the antioxidant activity of lime juice using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay

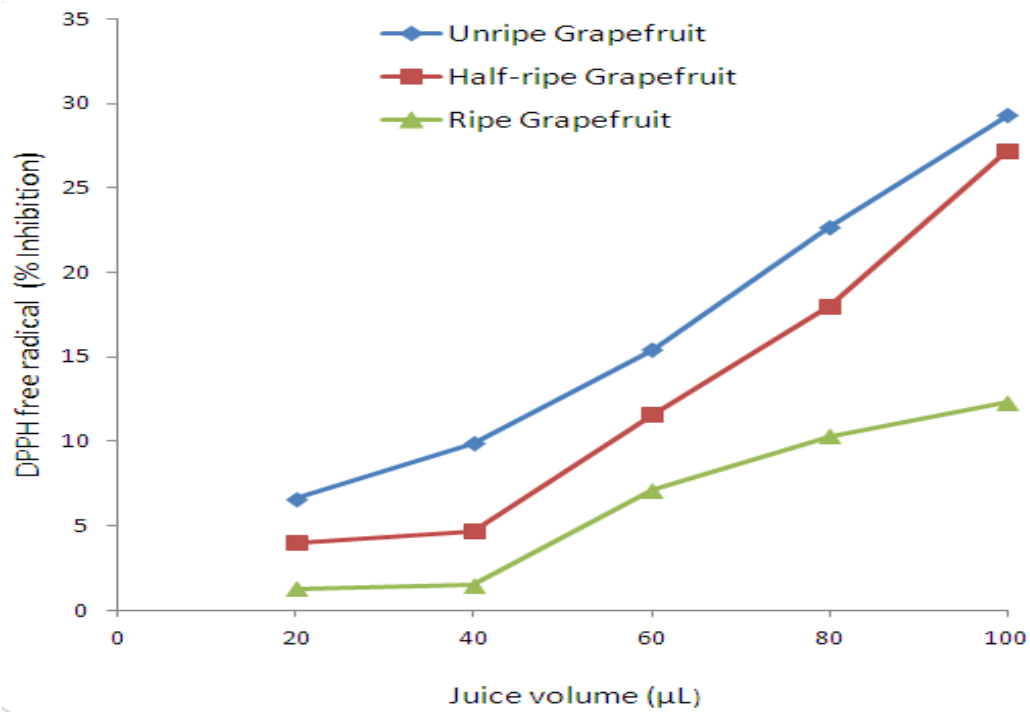

Fig. 3. Effect of degree of ripening and juice volume on the antioxidant activity of grapefruit juice using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay

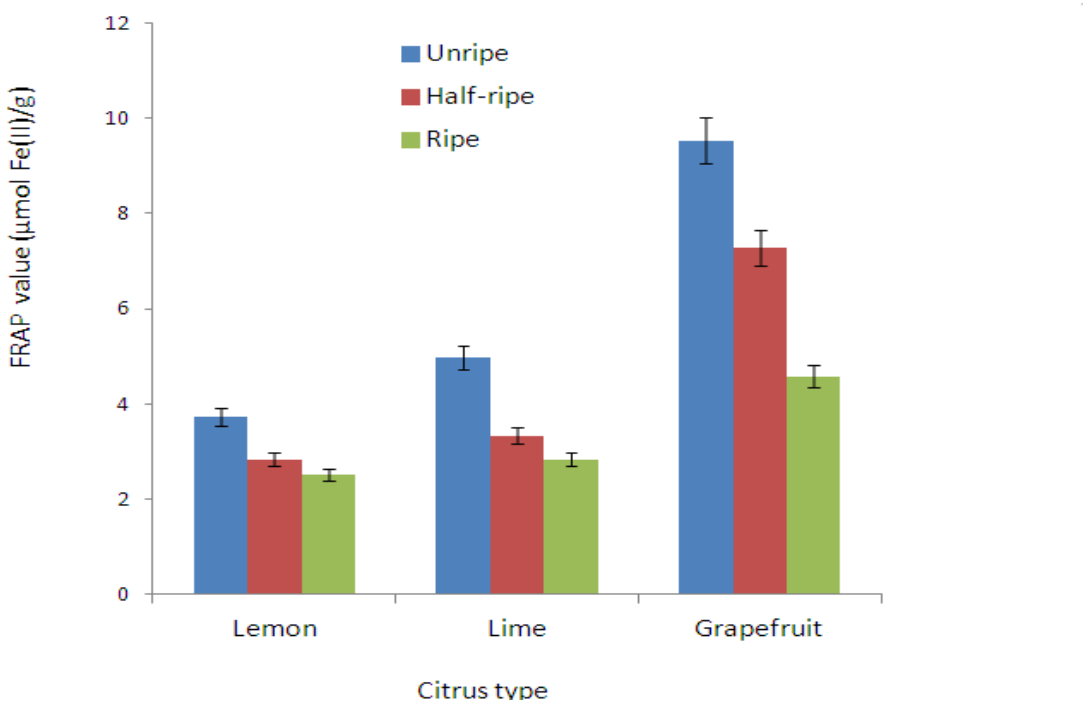

Fig. 4. Influence of degree of ripening on ferric-reducing antioxidant potential (FRAP) of juice from lemon, lime and grapefruit

The effect of degree of ripening and juice volume on the antioxidant activity of grapefruit juice using DPPH free radical assay is also presented in Fig. 3. The findings showed a general trend of percent inhibition of DPPH free radical by grapefruit juice decreasing with an increase in the level of ripening of the fruit. The value-ranges for percent inhibition of DPPH free radical by grapefruit juice, at different juice volumes, were 6.6–29.3% (unripe grapefruit), 4.0–27.2% (half-ripe grapefruit), and 1.3–12.3% (ripe grapefruits). It was similarly observed that the inhibition of DPPH free radical by grapefruit juice was dose-dependent as the inhibition of free radical was observed to be juice-volume dependent. At 20 µL juice volume, the percent inhibition was 6.6% (unripe), 4.0% (half-ripe), and 1.3% (ripe) while at 100 µL juice volume, the percent inhibition was 29.3% (unripe), 27.2% (half- ripe), and 12.3% (ripe). It was also observed that the trend of the free radical scavenging activity (percent inhibition) of grape fruit juice was similar to the trend of concentration of its biochemical constituents particularly ascorbic acid and total phenolic content. These findings have therefore indicated that the free radical scavenging activity of grapefruit juice could be influenced by both the degree of ripening and juice volume/concentration. Other influencing factors could be fruit variety and climate, among others [30,37].

3.4 Ferric-Reducing Antioxidant Potential (FRAP) of Juice from Lemon, Lime and Grapefruit as Influenced by Degree of Ripening

Fig. 4 shows the effect of degree of ripening on ferric-reducing antioxidant potential (FRAP) of juice from lemon, lime and grapefruit. The FRAP value was generally decreasing with an increase in the level of ripening while the value was also citrus-type-dependent as it showed the following pattern: grapefruit>lime>lemon. The FRAP values for lemon juice were 3.72 µ mol Fe(II)/g (unripe), 2.84 µ mol Fe(II)/g (half-ripe), and 2.51 µmolFe(II)/g (ripe). In the case of lime juice, the FRAP values were 4.98 µ mol Fe(II)/g (unripe), 3.34 µ mol Fe(II)/g (half-ripe), and 2.83 µmolFe(II)/g (ripe) while those of grapefruit juice were 9.53 µ mol Fe(II)/g (unripe), 7.28 µ mol Fe(II)/g (half-ripe), and 4.58 µ mol Fe(II)/g (ripe). The implication of these findings is that the juices from lemon, lime, and grapefruit have the potential to effect the reduction of Fe(III) ion to its lower valency of Fe(II) ion. The significance of this ferric-reducing antioxidant potential is that,

ordinarily, Fe(III) ion has been recognized to take part in the formation of reactive oxygen species (ROS) including free radicals thereby leading to metal-mediated lipid peroxidation, among others [38]. Therefore, the reduction of Fe(III) ion to a lower valency of Fe(II) ion will prevent the catalysis of formation reaction of ROS thereby serving as an anti-oxidation metallic ion [39,40].

4. CONCLUSION

This study has revealed that the degree of ripening of lemon (*Citrus limon*), lime (*Citrus aurantifolia*), and grapefruit (*Citrus paradisi*) had significant influence on their juice yield, biochemical characteristics and antioxidant properties. The juice yield was generally the highest when the fruits were at ripe stage; the pH and total acidity of the juices were influenced by level of ripening and fruit type while the ascorbic acid and total phenolic contents were also influenced by the level of ripening. The antioxidant capacity of the fruit juices on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and ferric-reducing antioxidant potential (FRAP) were reflections of the biochemical constituents of the individual fruits which were also influenced by the level of ripening and fruit-type.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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