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Anti oxidative potentials and storage stability of pasteurised mixed fruits juices from pineapple and bitter orange

Akeem Olayemi Raji [a,*], Olubumi Florence Adebayo [b], Saheed Mayowa Sanusi [c]

[a] Department of Food Science and Technology, Faculty of Agriculture, Kwara State University, Malete, Ilorin, Nigeria
[b] Product Development Programme, National Horticultural Research Institute, Ibadan, Oyo State, Nigeria
[c] Department of Food Engineering, University of Ilorin, Kwara State, Nigeria

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ABSTRACT

Humans are daily exposed to free radicals from various sources causing degenerative diseases, but can be prevented by daily intake of natural antioxidants. Bitter orange is an underutilized anti-oxidants source which can help in curbing degenerative diseases, if its consumption is encouraged by masking its bitterness or sourness with a natural sweetening pineapple. Pasteurized mixed fruits juices from various percentage of bitter orange and pineapple (0:100, 25:75, 50:50, 75:25 and 100:0%) were produced using standard procedures, stored at ambient condition and analyzed monthly for a period of six months. Their physicochemical properties, antioxidant activities, sensory properties and microbial load were carried out using standard procedures. Data obtained were analyzed using ANOVA. The pH, ^{o}brix, titratable acidity, vitamin C, total carotenoid, total antioxidant, DPPH, total phenolic, reducing power ranged from 2.43 to 4.34, 10.66–15.40 ^{o}brix, 0.08–0.86 mg/g, 0.10–0.45 mg/g, 0.03–0.98 mg/g, 0.25–1.01 mg/g, 14.95–91.28%, 0.10–0.28 mg/g, 0.11–1.64 mg/g respectively. The colour, taste, aroma, mouth feel and overall acceptability varied from 6.53 to 7.63, 4.42–8.16, 6.21–7.47, 4.63–7.89 and 5.21–7.89 respectively. The percentage of mixing and length of storage had significant influenced on all the aforementioned parameters at 95% confidence level. Juice sample having 25% of bitter orange and 75% pineapple was the most preferred by panelists, possessing high anti oxidants capacities and better keeping quality, while the quality of the product was acceptable up to 4 months without adding synthetic preservative at room temperature.

1. Introduction

Humans are virtually every day exposed to free radicals from ultraviolet (UV) light, cigarette smoke, ionizing radiation, certain organic solvents, pollutants and industrial waste (Boonchum et al., 2011). Free radicals are dangerous to health, causing several cellular damages (Park & Pezzuto, 2002). However, boosting the intake of naturally occurring antioxidants daily to curb degenerative diseases caused by frequent exposure to free radicals is highly important (Arueya et al., 2017). Recently, natural antioxidant compounds present in human diet have gained utmost attention as cancer-chemopreventive and chemotherapeutic agents.

Fruits and vegetables are very rich in phytochemicals, which include, resveratrol, ellagitannins, isothiocyanates, carotenoids, polyphenols, flavonoids and organosulfur compounds. The phytochemicals mentioned above had been reported to possess ability to prevent cardiovascular disease (Eggers & de Blanco, 2016). However, clinical finding on the cardiovascular health benefits of fruits and vegetables revealed that phytochemicals combination with fiber and other nutrients in whole food intake prevented oxidative damage and inflammation that might contribute to the pathogenesis of cardiovascular diseases (Ignarro et al., 2007). Regular consumption of fruit juices and pulps maintains health and makes up for the losses in the human diet (Costescu et al., 2006). The fruits antioxidant capacities make them as part of the most important foods for man (Kahkonen et al., 1999), their consumption are often for healthy nourishment and prevention of oxidative stress (Costescu et al., 2006).

Bitter orange (*Citrus aurantium)* fruit is a natural source of bioactive phytochemicals and it has been used for treatment some ailments (Pellati et al., 2002; Stohs et al., 2011). It possesses active compounds such as p-octopamin and synephrine alkaloids, which are good for human health and possess ability to mop off free radicals that cause health

* Corresponding author.
E-mail address: akeem.raji@kwasu.edu.ng (A.O. Raji).

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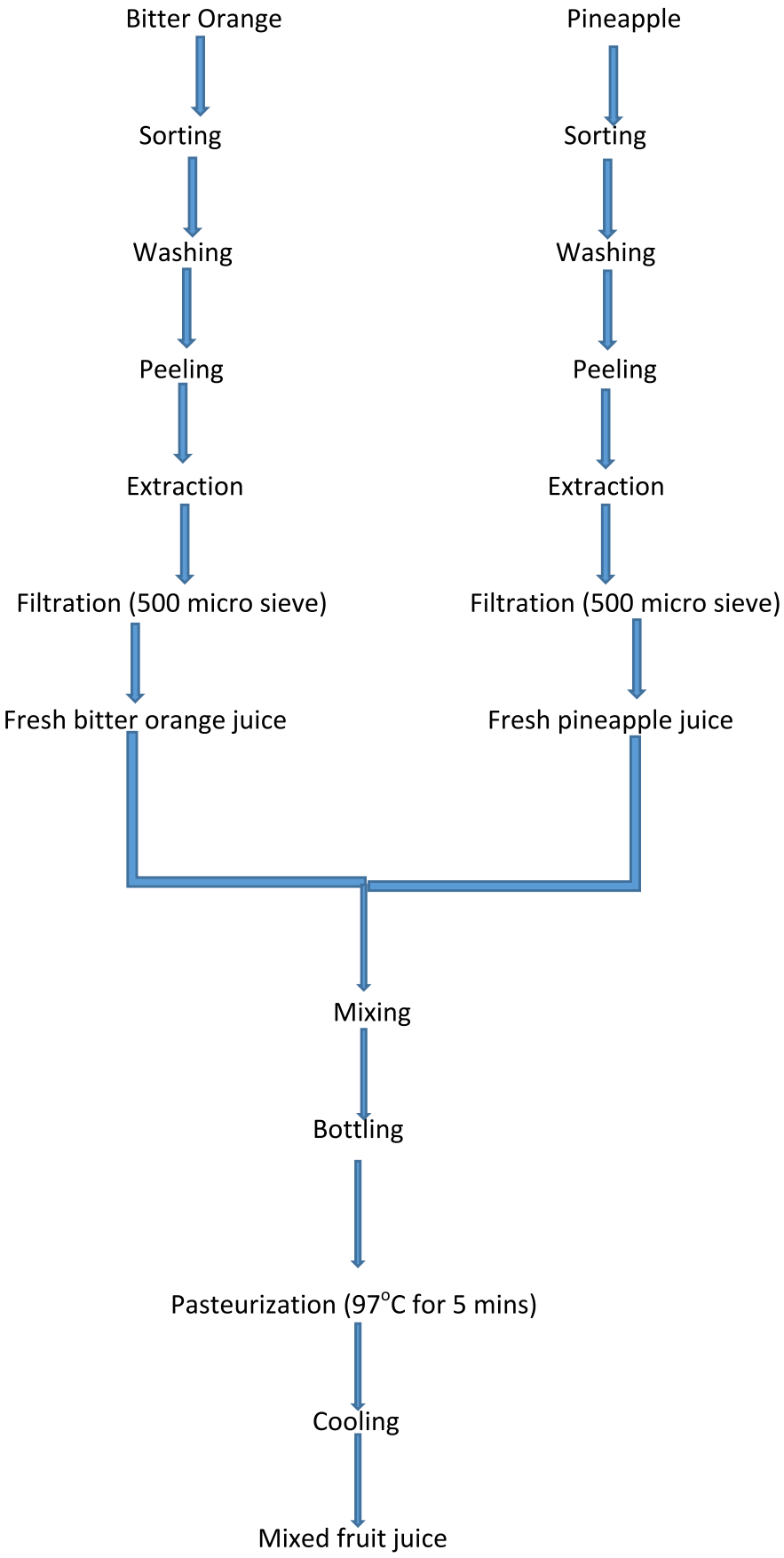


Fig. 1. Flow chart for the production of mixed fruit juice
Source: Cemeroglu (2004).

Table 1
Formulation for mixed fruit juice.

Products	Bitter Orange (%)	Pineapple (%)
100%J	100	0
100%J	0	100
75%J-25%P	75	25
50%J-50%P	50	50
25%J-75%P	25	75

problems (Pellati et al., 2002). *Citrus aurantium* fruit can be used for the treatment of inflammation, malarial fever, diarrhea and digestive disorder (Karthikeyan & Karthikeyan, 2014; Olowokudejo et al., 2008). However, bitter orange is an underutilized fruit with great potentials to curb degenerative diseases if it is incorporated in human diets, this can be achieved if its bitterness or sourness is masked with a natural sweetening fruit such as pineapple to encourage its consumption.

Pineapple (*Ananascomosus*) is a tropical non-citrus fruit that contains polyphenolics, attractive flavor and refreshing sugar-acid balance (Bartolome et al., 1995; Hossain & Rahman, 2011). It is being regarded as the most economically important plant in the family *Bromeliaceae* (Debnath et al., 2012). Pineapple fruit is a rich source of manganese and vitamins (Olanipekun et al., 2015). Pineapple fruit also contains some phytochemicals, most especially polyphenols which include gallic acid, coumaric acid, chlorogenic acid, epicatechin and arbutin (Ogawa et al. 2018).

Post-harvest losses and underutilization of tropical fruits and vegetables are of utmost concern for the development of fruits and vegetables Industries in Nigeria. Fruits are seasonal in nature and this has prompted many researchers to embark on findings on how to process and preserve fruit juices for usage during off-season (Oguntona & Akinyele, 1995; Tsegay et al., 2013). The fresh fruits shelf lives are limited by enzymatic and microorganism activities, and also due to the presence of osmophillic microflora (Islam et al., 2014). It is expected that an appropriate method of fruit preservation should be able to retain the original characteristics of fruit. Interestingly, thermal pasteurization is regarded as one of the most effective technologies used to extend food products shelf lives (Noci et al., 2008).

One of the underutilized fruits in Nigeria is bitter orange, despite its nutritional and medicinal benefits. However, consumption of foods dense in antioxidants is being increasingly known as an effective neutralizer of free radicals causing degenerative diseases (Moon & Shibamoto, 2009). Therefore, blending of bitter orange juice with pineapple juice in appropriate proportion might contribute to the sufficient intake of natural antioxidants. It might also discourage the use of synthetic preservatives in commercially available pasteurized fruit juices.

2. Materials and methods

2.1. Materials

2.1.1. Sample collection

Fully ripened and fresh bitter oranges (*Citrus aurantium)* and pineapple *(Ananascomosus)* fruits were collected on the 24th March 2020, from the orchard of National Horticultural Research Institute, Ibadan, Oyo State, Nigeria.

2.2. Methods

2.2.1. Preparation of juice

The fully ripened and fresh bitter orange and pineapple fruits were washed thoroughly with clean water to remove dirt. The cleaned pineapple and orange fruits were peeled, cut into pieces and the seeds in the orange fruits were removed. Their juices were extracted using locally fabricated multi-fruit juice extracting machine. The juices were filtered using a 500 micro aperture stainless steel screen (Fig. 1).

2.3. Experimental design

The fruit juice from pineapple was mixed with juice from bitter orange in percentage, ranging from 0 to 100% with 25% inclusion of bitter orange juice at intervals, summing up to five samples in total (Table 1). Samples were pasteurized at 97 °C for 5 min, and the temperature of each juice sample was monitored using a thermocouple during the heating process. The pasteurized juice samples were filled in sterilized glass brown bottles and capped with sterilized caps. All the samples were cooled and stored at room temperature for 180 days. Analyses were carried out once in every month on the stored samples.

2.4. Analyses on fruit juice samples

2.4.1. Physico-chemical analyses

The total soluble solids, pH, **vitamin C** and **titratable acidity contents of the juice samples were determined following the standard AOAC procedures** (AOAC, 2005).

2.4.2. Anti-oxidative potentials analyses

2.4.2.1. Total antioxidant capacity. The total antioxidant capacity was determined following the phosphate-molybdenum assay as described by Jayaprakasha et al. (2002). Precisely, concentrations of different 300 μl (30, 60,90,120 μg/ml) of the extract (juice) and standard (ascorbic acid and rutin) were mixed together with 3 ml of a reagent solution which contains 0.6 m MH_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate in test tubes. The test tubes covered with aluminum foil were incubated in a boiling water bath at 95 °C for 90 min. A blank was prepared with 3 mL of the reagent solution and 300 μL of distilled water. The blank was also incubated in a boiling water bath at the same condition stated above. The incubated samples were then allowed to cool to room temperature and the absorbance from each sample was measured at 695 nm against the blank.

2.4.2.2. Ferric reducing power. The Ferric reducing power of each sample was established by determining the ability of each sample to reduce FeCl solution as described by Pulido et al.(2000). One milliliter of the aliquot of each sample (0.5 ml of each sample of the juice homogenized with 20 ml ethanol) was added to a solution of 2.5 ml of 200 Mm sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture obtained was incubated at 50 °C for 20mins. After incubation, 2.5 ml of 10%Trichloroacetic acid was introduced into the mixture and centrifuged at 650 rpm for 10min. The supernatant (2.5 ml) was introduced into a solution containing 2.5 ml of de-ionized water and 0.5 ml of ferric chloride. The absorbance was later read at 700 nm and compared with the catechin standard.

2.4.2.3. Free radical scavenging ability. The samples' DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical scavenging abilities was evaluated following the method of Ursini*et al.* (1994). Precisely, 0.2 ml of aliquot of each juice sample (0.5 ml of each sample of the juice homogenized with 10 ml methanol) was added to a solution of 7.6 ml of 0.4 Mm methanol containing DPPH (1 mM). A milliliter (1 ml) of each resulting mixture was shaken vigorously and left to stand for 30 min at ambient temperature. The catechin (standard) and blank were also prepared at similar concentrations using methanol and DPPH. Thereafter, resulting mixtures from the samples and standard were incubated in a water bath at 37 °C for 30 min, and their absorbance were read against the blank at 517 nm. The percent inhibition was expressed as:

$$\% \text{ Inhibition} = [(C–S)/C] \times 100$$

Where S = absorbance of the sample and C = absorbance of the blank.

The 50% inhibitory concentration (IC_{50}) was calculated as the quantity of the extract needed to react with one and a half of DPPH radicals.

2.4.2.4. Total phenolic content. The total phenolic content of each juice extract was determined according to the modified method described by Kaur and Kapoor (2002). A crude extract (200 μL) of the juice (1 mg/mL) was made up to 3 mL with distilled water and it was mixed vigorously with 0.5 mL of Folin-Ciocalteu reagent for 3 min, after which 2 ml of 20% (w/v) sodium carbonate was added to the mixture. The overall mixture was allowed to stand for a further 60 min in the dark, while the absorbance was read at 650 nm in UV–visible spectrophotometer (Spectrum lab 752s). The procedure was repeated for the standard (catechin) and the results were obtained in three replicate.

2.4.2.5. Total flavonoids content. Total flavonoids content were measured using Aluminium chloride colorimetric method as described by Zhishen et al. (1999). One milliliter of the aliquot of each juice sample and 1 mL of aliquot catechin (standard) at different concentrations was added to a 10 ml volumetric flask containing 4 ml de-ionized water. Subsequently, 0.3 ml of 10% Aluminium chloride was added. Six minutes after, 2 ml of 1M NaOH was introduced to the mixture; the solution was diluted immediately to volume (10 ml) with de-ionized water and thoroughly mixed thereafter. The absorbance was read at 510 nm with a spectrophotometer and compared with the standard.

2.4.2.6. Determination of total carotenoid. The total carotenoid contents of the juice samples were determined following the method described by Velioglu et al. (1998). One milliliter of the aliquot of each juice sample and 10 ml of n-hexane –acetone –ethanol (v/v; 2:1:1) were poured into a flask and extracted on a shaker at 200 rpm for 20mins at ambient temperature. The mixture was centrifuged at 4000 rpm for 10min at 4 °C. The supernatant obtained was made up to 20 ml with the extraction solvents. Two layers were noticed after mixing, an organic (upper layer) and aqueous (lower layer). After careful separation of layers, the absorbance the upper layer was read at 450 nm.

2.5. Microbiological analyses

Enumeration of bacteria in juice samples was carried out using conventional colony count method as described by Jasson et al. (2010). One milliliter of each juice sample was decimally diluted and 0.1 mL of 10-1 dilutions was inoculated on nutrient agar plates and incubated at 37 °C for 24h. After incubation, colony counter (Subra Scientific Co., India) was used to enumerate the observed colonies. The dilution factors were taken into consideration in the final counts, thus providing the initial number of the cells. Analysis was replicated three times and the arithmetic mean was reported as the final result.

2.5.1. Fungi counts

The fungi count was carried out using the Raper and Fennell (1977) method as reported by Atanda et al. (2009). One mililitre of each sample was transferred aseptically into 9 mL distilled water and it was homogenized adequately. Subsequently, the samples were decimally diluted, and all the samples were analyzed in three replicate on potato dextrose agar containing 0.001% of chloraphenicol. The agars containing the inoculums were allowed to incubate at ambient temperature (30 °C) for 48 h. After incubation, colony counter (Subra Scientific Co., India) was used to enumerate the observed colonies and the arithmetic mean was reported as the final result.

2.6. Sensory analysis

Panel of twenty judges were selected from the postgraduate students of Kwara State University and staff of the National Horticultural Research Institute, Ibadan, Nigeria, who were familiar with fruits drinks organoleptic assessments. Sensory evaluation (color, flavor, taste and overall acceptability) of mixed juice was carried out using a 9 point hedonic scale in a well-ventilated sensory evaluation room at 25 °C. The hedonic scale was ranked as; like extremely to very much (8–9 scores), like moderately to like slightly (5–7 scores), neither like nor dislike to dislike slightly or dislike moderately (2–4 scores) and dislike extremely to dislike very much (0–1 score). Each panelist assessed an average of 5 mL of juice, which were served in 25 mL plastic cups coded with 3 digits in a monadic manner and in a balanced order. The sensory evaluation room was well ventilated at 25 °C.

Table 2
Sensory evaluation of freshly prepared mixed fruit juices obtained from bitter orange and pineapple fruits.

Sample	Colour	Taste	Aroma	Mouth Feel	Overall Acceptability
Fresh					
100%J	7.16 ± 1.50^{ab}	7.47 ± 1.47^{ab}	7.37 ± 1.21^{a}	7.84 ± 1.12^{a}	7.79 ± 0.86^{a}
100% P	6.53 ± 1.68^{b}	4.42 ± 1.84^{c}	6.21 ± 1.58^{b}	4.63 ± 2.06^{c}	5.21 ± 2.07^{c}
75% J:25% P	6.63 ± 1.64^{b}	5.16 ± 2.12^{c}	6.26 ± 1.52^{b}	5.16 ± 2.57^{c}	5.47 ± 2.34^{bc}
50%J: 50%P	7.21 ± 1.03^{ab}	6.53 ± 1.74^{b}	6.95 ± 1.08^{ab}	6.47 ± 1.26^{b}	6.42 ± 1.35^{b}
25%J: 75%P	7.63 ± 0.90^{a}	8.16 ± 1.17^{a}	8.16 ± 1.17^{a}	7.89 ± 0.94^{a}	7.89 ± 1.00^{a}
1 Month					
100%J	7.15 ± 1.41^{ab}	7.40 ± 1.37^{ab}	7.35 ± 1.20^{a}	7.84 ± 1.00^{a}	7.78 ± 0.85^{a}
100% P	6.53 ± 1.61^{b}	4.40 ± 1.32^{c}	6.21 ± 1.18^{b}	4.60 ± 2.16^{c}	5.20 ± 2.17^{c}
75% J:25% P	6.63 ± 1.44^{b}	5.13 ± 2.02^{c}	6.25 ± 1.53^{b}	5.15 ± 2.57^{c}	5.44 ± 2.33^{bc}
50%J: 50%P	7.20 ± 1.53^{ab}	6.52 ± 1.70^{b}	6.95 ± 1.01^{ab}	6.46 ± 1.27^{b}	6.41 ± 1.34^{b}
25%J: 75%P	7.61 ± 0.39^{a}	8.11 ± 1.14^{a}	8.15 ± 1.17^{a}	7.88 ± 0.94^{a}	7.85 ± 0.99^{a}
2 Months					
100%J	7.14 ± 1.31^{ab}	7.37 ± 1.465^{ab}	7.32 ± 1.21^{a}	7.82 ± 1.22^{a}	7.75 ± 0.86^{a}
100% P	6.52 ± 1.60^{b}	4.36 ± 1.385^{c}	6.20 ± 1.58^{b}	4.60 ± 2.06^{c}	5.18 ± 2.24^{c}
75% J:25% P	6.62 ± 1.54^{b}	5.10 ± 2.001^{c}	6.25 ± 1.02^{b}	5.15 ± 2.17^{c}	5.43 ± 2.44^{bc}
50%J: 50%P	7.20 ± 1.08^{ab}	6.50 ± 1.64^{b}	6.93 ± 1.18^{ab}	6.44 ± 1.29^{b}	6.38 ± 1.45^{b}
25%J: 75%P	7.58 ± 0.80^{a}	8.07 ± 1.107^{a}	8.13 ± 1.27^{a}	7.87 ± 0.74^{a}	7.82 ± 0.65^{a}
3 Months					
100%J	7.13 ± 1.31^{ab}	7.33 ± 1.76^{ab}	7.30 ± 1.11^{a}	7.81 ± 1.02^{a}	7.74 ± 0.85^{a}
100% P	6.52 ± 1.58^{b}	4.30 ± 1.87^{c}	6.19 ± 1.56^{b}	4.659 ± 2.07^{c}	5.16 ± 2.19^{c}
75% J:25% P	6.62 ± 1.06^{b}	5.06 ± 1.92^{c}	6.24 ± 1.62^{b}	5.13 ± 2.27^{c}	5.40 ± 2.39^{bc}
50%J: 50%P	7.19 ± 1.73^{ab}	6.46 ± 1.60^{b}	6.92 ± 1.01^{ab}	6.41 ± 1.36^{b}	6.37 ± 1.15^{b}
25%J: 75%P	7.15 ± 0.96^{a}	8.04 ± 1.07^{a}	8.11 ± 1.12^{a}	7.85 ± 0.64^{a}	7.79 ± 0.90^{a}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p < 0.05$).
Keys;
100J-100% Bitter orange juice.
100P-100% Pineapple juice.
75J: 25P-75% Bitter orange & 25% pineapple juice.
50J:50P-50% Bitter orange & 50% pineapple juice.
25J:75P-25% Bitter orange& 75% pineapple juice.

Table 3
Influence of ambient storage on the physico-chemical properties of fruit juices.

Samples/ Months	^{o} Brix	pH	Titratable acidity (mg/100 ml)	Vitamin C (mg/ml)
100% J				
Fresh	14.30 ± 0.52^{ab}	3.88 ± 0.01^{g}	0.29 ± 0.02^{p}	0.45 ± 0.11^{a}
1Month	14.30 ± 0.52^{a}	3.81 ± 0.01^{a}	0.25 ± 0.00^{a}	0.45 ± 0.11^{c-e}
2 Months	14.20 ± 0.61^{a}	3.24 ± 0.01^{e}	0.25 ± 0.00^{h}	0.44 ± 0.11^{a}
3 Months	14.13 ± 0.12^{a}	3.14 ± 0.04^{e}	0.24 ± 0.02^{i}	0.43 ± 0.10^{a}
4 Months	14.00 ± 0.00^{a}	3.00 ± 0.01^{b}	0.21 ± 0.01^{ab}	0.29 ± 0.07^{cd}
5 Months	13.16 ± 0.29^{ab}	3.00 ± 0.01^{h}	0.18 ± 0.02^{q}	0.22 ± 0.05^{a}
6 Months	13.06 ± 0.12^{a}	2.43 ± 0.00^{b}	0.08 ± 0.00^{a}	0.18 ± 0.04^{c-e}
100% P				
Fresh	15.40 ± 0.00^{ab}	4.34 ± 0.02^{h}	0.86 ± 0.03^{n}	0.25 ± 0.04^{a}
1 Month	14.66 ± 0.58^{ab}	4.23 ± 0.01^{f}	0.84 ± 0.00^{ij}	0.25 ± 0.04^{ab}
2 Months	15.13 ± 0.12^{a}	4.08 ± 0.00^{c}	0.85 ± 0.02^{ef}	0.25 ± 0.04^{cd}
3 Months	15.20 ± 0.00^{ab}	3.99 ± 0.01^{o}	0.80 ± 0.03^{q}	0.24 ± 0.04^{a}
4 Months	15.40 ± 0.36^{a}	3.49 ± 0.04^{d}	0.79 ± 0.02^{a}	0.16 ± 0.03^{c-e}
5 Months	14.66 ± 0.58^{ab}	3.35 ± 0.02^{n}	0.77 ± 0.02^{lm}	0.12 ± 0.02^{a}
6 Months	12.06 ± 1.79^{ab}	2.91 ± 0.01^{l}	0.73 ± 0.00^{i}	0.10 ± 0.02^{ab}
75% J: 25% P				
Fresh	14.60 ± 0.00^{a}	3.98 ± 0.01^{i}	0.61 ± 0.02^{de}	0.45 ± 0.04^{cd}
1 Month	14.01 ± 0.03^{ab}	3.78 ± 0.01^{q}	0.39 ± 0.00^{qr}	0.44 ± 0.04^{a}
2 Months	14.00 ± 0.00^{a}	3.30 ± 0.00^{e}	0.43 ± 0.02^{bc}	0.44 ± 0.04^{c-e}
3 Months	14.00 ± 0.00^{ab}	3.22 ± 0.02^{o}	0.35 ± 0.02^{o}	0.42 ± 0.04^{a}
4 Months	14.00 ± 0.00^{ab}	3.13 ± 0.04^{n}	0.35 ± 0.03^{j}	0.29 ± 0.03^{ab}
5 Months	10.86 ± 6.14^{a}	3.03 ± 0.04^{j}	0.26 ± 0.02^{g}	0.22 ± 0.02^{c-e}
6 Months	10.66 ± 1.16^{ab}	2.52 ± 0.02^{r}	0.26 ± 0.02^{r}	0.18 ± 0.02^{bc}
50% J: 50% P				
Fresh	14.90 ± 0.00^{a}	4.01 ± 0.03^{k}	0.56 ± 0.00^{cd}	0.39 ± 0.01^{d-g}
1 Month	14.13 ± 0.12^{ab}	3.93 ± 0.02^{q}	0.54 ± 0.03^{o}	0.38 ± 0.01^{bc}
2 Months	14.00 ± 0.00^{a-c}	3.38 ± 0.01^{pq}	0.56 ± 0.00^{k}	0.38 ± 0.01^{c-e}
3 Months	14.00 ± 0.00^{a}	3.30 ± 0.02^{i}	0.52 ± 0.02^{g}	0.37 ± 0.01^{d-g}
4 Months	13.63 ± 0.55^{a-c}	3.17 ± 0.01^{p}	0.49 ± 0.02^{s}	0.25 ± 0.01^{c-f}
5 Months	13.63 ± 0.55^{a}	3.34 ± 0.02^{lm}	0.45 ± 0.00^{de}	0.19 ± 0.01^{fg}
6 Months	12.00 ± 1.73^{d}	3.17 ± 0.01^{r}	0.40 ± 0.02^{pq}	0.15 ± 0.00^{c-f}
25% J: 75% P				
Fresh	15.00 ± 0.00^{a-c}	4.24 ± 0.02^{mn}	0.83 ± 0.03^{l}	0.26 ± 0.07^{d-g}
1 Month	15.00 ± 0.00^{ab}	4.14 ± 0.01^{lm}	0.74 ± 0.02^{h}	0.26 ± 0.07^{fg}
2 Months	14.87 ± 0.12^{a-c}	3.57 ± 0.06^{w}	0.77 ± 0.02^{t}	0.25 ± 0.07^{d-g}
3 Months	14.60 ± 0.52^{b-d}	3.53 ± 0.01^{s}	0.67 ± 0.05^{f}	0.25 ± 0.07^{g}
4 Months	14.60 ± 0.52^{d}	3.56 ± 0.02^{v}	0.69 ± 0.02^{pq}	0.17 ± 0.05^{d-g}
5 Months	14.16 ± 0.29^{b-d}	3.36 ± 0.03^{u}	0.62 ± 0.00^{mn}	0.13 ± 0.03^{a-g}
6 Months	11.66 ± 0.58^{cd}	2.87 ± 0.02^{t}	0.55 ± 0.02^{ij}	0.10 ± 0.03^{g}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p < 0.05$).

2.7. Statistical analysis

Data collected were subjected to analysis of variance [ANOVA] at $p < 0.05$ using SPSS [Version 17.0, 2002] statistical package, and their means were separated with Duncan Multiple Range Test (DMRT) according to Larmond (1977, pp. 1637–1674).

3. Result and discussion

3.1. Sensory evaluation of the mixed fruit juice

Table 2 shows the mean sensory scores of the blended juice, processed from bitter orange and pineapple fruits. The evaluations were done for colour, aroma, mouth feel, taste and overall acceptability and these parameters ranged from 6.53 to 7.63, 4.42–8.16, 6.21–7.47, 4.63–7.89 and 5.21–7.89 respectively. Sample 25J:75 P was mostly preferred in terms of colour, aroma, mouth feel, taste and overall acceptability. Least preference was given to the 100% pineapple juice. The sensory ratings for colour of the juice samples was similar to the range of 5.14–8.35 Ndife et al. (2013) reported for different brands of orange juice samples, and slight reduction in the colour ratings might be as a result of an increase in non-enzymatic browning reaction of fruit juice during storage (Singh & Sharma, 2017). However, it was observed that as the ratio of bitter orange inclusion increased beyond 25%, the sensory properties decreased. Organoleptic parameters like color, flavor, and taste of the juices were acceptable up to 90 days of storage, except for sample 100P (100% pineapple) that developed a change in color and possessed an objectionable taste after 90 days. However, sensory characteristics are of great importance in consumer's point of view (Ghadge et al., 2008), the mixed fruit are expected to possess good sensory attributes on the basis of overall acceptability. The values reported for overall acceptability of the mixed fruit samples at different ratios fell within the like region, but slightly declined as the storage period increased. This was in accordance with the findings of Banigo et al. (2015) who reported an acceptable sensory scores range of 6.05–7.80 on a 9-point hedonic scale for soy/carrot/beetroot juice, and Emelike and Ebere (2016) who established a range of 3.50–4.56 on a 5 – point hedonic scale for cashew apple juice. Judging from the ratings of the panelists, sample with 25% of bitter orange and 75% pineapple was the most preferred in terms of overall acceptability, and these imply that bitter orange that is an underutilized crop can now be incorporated up to 25% in pineapple based mixed fruit juice production.

3.2. The physico-chemical properties of mixed fruit juices

The results of the findings of physico-chemical properties of mixed fruit juices are shown in Table 3. The mixing ratio and the length of storage significantly influenced the physico-chemical properties of mixed fruit juices.

3.2.1. pH

The pH values of the mixed fruit juices ranged from 2.43 to 4.34 (Table 3). The pH is one of the important quality characteristics that depict the stability of bioactive compounds in fruit juice (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006). There was a significant decrease in pH during storage, which affected the organoleptic quality of juice. During storage, it was observed that as there was an inverse relationship between the storage period and the pH of the juice samples. Reduction in pH at ambient storage condition might indicate proliferation of acid tolerant microorganisms, which could produce spores or toxins that might lower the pH of the juice samples (Suriati et al., 2020). The pH of the juices samples after four months of storage were within the recommended range of 3–5 reported by Harris et al.

Table 4
Influence of ambient storage on the anti oxidative properties of the mixed fruit juices.

Sample/Months	Total Phenolic (mg/ml)	Flavonoid (mg/ml)	DPPH (%)	Reducing power (mg/ml)	Total Antioxidant (mg/ml)	Total Carotenoid (mg/ml)
100% J						
Fresh	0.28 ± 0.02^{a}	0.28 ± 0.03^{a}	90.33 ± 0.47^{a}	1.64 ± 0.03^{a}	0.82 ± 0.02^{cd}	0.98 ± 0.00^{a}
1Month	0.24 ± 0.00^{ef}	$0.19 \pm 0.00^{i-l}$	84.84 ± 0.74^{ef}	1.34 ± 0.03^{c}	0.69 ± 0.04^{a}	0.47 ± 0.00^{m}
2 Months	0.24 ± 0.02^{a}	0.27 ± 0.01^{cd}	66.36 ± 3.88^{a}	1.12 ± 0.03^{b}	0.59 ± 0.04^{b}	0.34 ± 0.01^{b}
3 Months	0.22 ± 0.00^{a}	0.25 ± 0.01^{ef}	64.37 ± 1.15^{cd}	1.00 ± 0.03^{b}	0.51 ± 0.02^{a}	0.45 ± 0.00^{c}
4 Months	0.20 ± 0.00^{cd}	0.14 ± 0.00^{de}	$49.39 \pm 1.79^{c-e}$	0.97 ± 0.03^{c}	0.67 ± 0.03^{a}	0.39 ± 0.03^{fg}
5 Months	0.16 ± 0.00^{bcd}	0.07 ± 0.01^{fg}	46.29 ± 5.01^{b}	0.75 ± 0.03^{b}	0.34 ± 0.01^{ef}	0.39 ± 0.00^{d}
6 Months	0.14 ± 0.00^{ghi}	0.06 ± 0.01^{gh}	$23.62 \pm 0.87^{f-h}$	0.37 ± 0.02^{fg}	0.25 ± 0.01^{bc}	0.17 ± 0.05^{o}
100% P						
Fresh	0.21 ± 0.01^{b}	$0.14 \pm 0.02^{k-m}$	73.99 ± 2.08^{ab}	1.11 ± 0.07^{cd}	1.01 ± 0.02^{d}	0.15 ± 0.00^{d}
1 Month	$0.19 \pm 0–01^{b}$	0.18 ± 0.02^{lm}	70.47 ± 2.80^{fg}	$0.81 \pm 0.07^{c-e}$	0.87 ± 0.08^{e}	0.07 ± 0.00^{i}
2 Months	0.19 ± 0.01^{cd}	0.15 ± 0.02^{mn}	$58.30 \pm 0.69^{g-i}$	0.59 ± 0.07^{f}	$0.81 \pm 0.04^{b-d}$	0.07 ± 0.00^{lm}
3 Months	0.16 ± 0.01^{bcd}	0.14 ± 0.02^{ab}	$55.05 \pm 2.98^{h-j}$	0.47 ± 0.07^{c}	0.70 ± 0.06^{gh}	0.06 ± 0.01^{h}
4 Months	0.15 ± 0.01^{ghi}	$0.08 \pm 0.01^{h-j}$	40.09 ± 0.92^{kl}	0.44 ± 0.07^{jk}	0.69 ± 0.01^{cd}	0.05 ± 0.00^{op}
5 Months	0.12 ± 0.01^{bc}	0.03 ± 0.01^{hi}	42.77 ± 4.18^{bc}	0.23 ± 0.072^{f}	0.45 ± 0.05^{eg}	0.04 ± 0.01^{e}
6 Months	0.10 ± 0.01^{de}	$0.02 \pm 0.01^{j-m}$	$14.95 \pm 0.92^{d-f}$	0.11 ± 0.04^{fg}	0.28 ± 0.02^{eg}	0.03 ± 0.01^{i}
75% J: 25% P						
Fresh	0.28 ± 0.01^{fgh}	$0.24 \pm 0.01^{i-l}$	$91.28 \pm 12.32^{h-j}$	1.39 ± 0.09^{ij}	0.89 ± 0.04^{cd}	0.77 ± 0.00^{lm}
1 Month	0.25 ± 0.01^{de}	0.12 ± 0.01^{bc}	86.95 ± 1.25^{ij}	1.09 ± 0.09^{de}	0.78 ± 0.00^{i}	0.45 ± 0.01^{d}
2 Months	0.24 ± 0.01^{jkl}	$0.16 \pm 0.03^{i-l}$	83.23 ± 3.58^{lm}	0.86 ± 0.09^{lm}	0.64 ± 0.03^{e}	$0.41 \pm 0.00^{o-r}$
3 Months	0.18 ± 0.00^{hij}	$0.15 \pm 0.02^{i-k}$	83.19 ± 2.16^{bc}	$0.75 \pm 0.09^{f-h}$	0.56 ± 0.00^{hi}	0.35 ± 0.00^{gh}
4 Months	0.16 ± 0.00^{fgh}	0.09 ± 0.01^{lm}	$67.51 \pm 2.79^{h-j}$	$0.72 \pm 0.09^{g-i}$	0.63 ± 0.01^{gh}	0.37 ± 0.06^{j}
5 Months	0.13 ± 0.00^{ijk}	$0.06 \pm 0.01^{j-m}$	66.84 ± 1.73^{jk}	0.50 ± 0.09^{kl}	0.35 ± 0.01^{e}	0.19 ± 0.00^{m}
6 Months	0.11 ± 0.00^{fg}	$0.05 \pm 0.01^{i-l}$	19.43 ± 1.51^{no}	0.24 ± 0.05^{e}	0.27 ± 0.01^{ef}	0.13 ± 0.01^{ef}
50% J: 50% P						
Fresh	0.27 ± 0.03^{lm}	$0.21 \pm 0.02^{n-p}$	79.42 ± 0.92^{q}	1.35 ± 0.07^{lm}	1.00 ± 0.02^{ef}	$0.56 \pm 0.00^{o-r}$
1 Month	0.25 ± 0.01^{jkl}	0.12 ± 0.03^{no}	$72.43 \pm 1.78^{g-j}$	$1.05 \pm 0.07^{g-i}$	0.70 ± 0.01^{fg}	0.30 ± 0.01^{fg}
2 Months	0.22 ± 0.00^{hij}	$0.13 \pm 0.02^{o-q}$	$74.22 \pm 0.71^{l-n}$	$0.82 \pm 0.07^{h-j}$	0.65 ± 0.03^{ij}	0.30 ± 0.02^{k}
3 Months	0.20 ± 0.01^{klm}	0.12 ± 0.02^{op}	$64.90 \pm 1.99^{m-o}$	0.71 ± 0.07^{kl}	0.60 ± 0.02^{fg}	0.26 ± 0.01^{n}
4 Months	0.18 ± 0.01^{klm}	$0.07 \pm 0.01^{o-q}$	53.68 ± 0.70^{op}	$0.68 \pm 0.07^{f-h}$	0.50 ± 0.03^{lm}	0.22 ± 0.01^{ef}
5 Months	0.14 ± 0.01^{pqr}	$0.03 \pm 0.01^{s-u}$	42.89 ± 2.82^{pq}	0.46 ± 0.07^{op}	0.37 ± 0.01^{jk}	$0.17 \pm 0.00^{o-q}$
6 Months	0.12 ± 0.01^{nop}	$0.01 \pm 0.01^{p-r}$	$16.19 \pm 1.00^{h-j}$	0.22 ± 0.03^{kl}	0.28 ± 0.03^{l}	0.04 ± 0.01^{l}
25% J: 75% P						
Fresh	0.23 ± 0.01^{mno}	$0.22 \pm 0.02^{t-u}$	78.82 ± 1.84^{pq}	1.16 ± 0.05^{lm}	0.96 ± 0.08^{l}	0.37 ± 0.00^{lm}
1 Month	0.22 ± 0.01^{opq}	$0.11 \pm 0.01^{r-u}$	68.60 ± 0.10^{no}	0.86 ± 0.05^{no}	0.87 ± 0.06^{kl}	0.17 ± 0.01^{n}
2 Months	0.19 ± 0.00^{mn}	$0.14 \pm 0.01^{p-s}$	66.46 ± 0.36^{r}	0.63 ± 0.05^{mn}	0.82 ± 0.08^{n}	0.18 ± 0.01^{lm}
3 Months	0.17 ± 0.01^{r}	$0.13 \pm 0.01^{t-u}$	62.75 ± 3.57^{s}	0.52 ± 0.05^{q}	0.70 ± 0.06^{mn}	$0.16 \pm 0.01^{o-r}$
4 Months	0.16 ± 0.01^{pqr}	$0.08 \pm 0.00^{q-t}$	50.09 ± 4.48^{s}	0.49 ± 0.05^{o}	0.63 ± 0.01^{n}	0.13 ± 0.00^{r}
5 Months	0.12 ± 0.01^{nopq}	0.04 ± 0.01^{u}	49.08 ± 0.46^{s}	0.27 ± 0.05^{op}	0.40 ± 0.01^{mn}	$0.12 \pm 0.00^{p-r}$
6 Months	0.11 ± 0.01^{qr}	0.02 ± 0.01^{tu}	21.43 ± 0.86^{r}	0.13 ± 0.02^{pq}	0.28 ± 0.01^{mn}	0.04 ± 0.01^{qr}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p < 0.05$).

(1991, pp. 63–68) and Akusu et al. (2016) for fruits and vegetables, but fell below 3 after five months of storage. Similar results were reported by Dhaliwal and HIRA (2001) and Cortes et al. (2008) who observed that the decrease in pH values of fruit juices during storage which were caused by the spoilage microorganisms.

3.2.2. Titratable acidity

The titratable acidity values of the mixed fruit juices ranged from 0.08 to 0.86mg/100 ml (Table 3). The titratable acidity (TTA) was established to determine the degree of acidity of fruit juices caused by acid production by polysaccharides and oxidation of reducing sugar during maturity process. It has an indirect relationship with the pH of fruit juice and can contribute to fruit acidity (Akusu et al., 2016; Sadler & Murphy, 2010). However, the storage period had less significant effect on the TTA values obtained in this study, but TTA values were significantly influenced by the mixing ratio. The TTA values increased as the percentage of pineapple juice increased in the mixed fruit juice.

3.2.3. Vitamin C content

The vitamin C content of the mixed fruit juice ranged from 0.10 to 0.45 mg/ml (Table 3). It was observed that the freshly prepared 25% bitter orange and 75% pineapple juice sample had the lowest value of 0.26 mg/ml and decreased to 0.10 mg/ml after six months of storage, while both freshly prepared 100% bitter orange juice and 25%:75% (pineapple: bitter orange)juice samples had the same highest value of 0.45 mg/ml, which eventually decreased to 0.18 mg/ml in both samples. Ascorbic acid (vitamin C) is an important nutrient that possesses antioxidant ability and provides protection against free radicals (Esteve et al., 2005). It is also considered as an indicator of the nutritional quality of juices (Bull et al., 2004). Storage temperature, type of processing and packaging materials affect the rate of ascorbic acid degradation during storage (Ayhan et al., 2001). However, decrease in vitamin C contents were observed in all the juice samples during storage and this might be due to the oxidative sensitivity of vitamin C to oxygen, light and heat, aided by both enzymatic and non-enzymatic catalysts (Davey et al., 2000). Thermally pasteurized juice is expected to retain 50% of their initial ascorbic acid up to 4 months. Juices that possess values that are lesser than 50% of their initial Vitamin C contents during storage are considered to be unsafe (Pérez et al., 2005). Interestingly, the vitamin C contents of the juices samples after four months of storage were above 50% of their initial contents, but fell below 50% of their initial contents after five months of storage. This implies that their quality cannot be guaranteed after four months of storage.

3.2.4. Brix content

The brix content of the mixed fruit juice decreased from 15.40 to 10.66 ^{o}Brix (Table 3). The fresh pineapple juice sample (100%) had the highest value, while juice sample that contained 25% bitter orange and that had been stored for six months had the lowest value. The values obtained were higher than the values (5.50–11.80%) Ndife et al. (2013) reported different brands of orange juice samples, but was in close relationship with the range of 10.20–14.88% which Akusu et al. (2016)

reported for orange/pineapple fruit juice blends. It was observed that the brix contents of the juice samples decreased as the storage time increased. However, the decrease in the brix contents during storage might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. Similar finding was reported by Bull et al. (2004) for thermally processed valencia and navel orange juice stored at room temperature.

3.3. The antioxidant properties of mixed fruit juices under ambient storage

The Influence of ambient storage on the anti-oxidative properties of the mixed fruit juices is presented in Table 4.

3.3.1. Total flavonoids

The total flavonoids values of the mixed fruit juices ranged from 0.01 to 0.24 mg/ml (Table 4). Freshly prepared 100% bitter orange had the highest value (0.28 mg/ml), while juice sample that contained 50% bitter orange and that had been stored for six months had the lowest value (0.01 mg/ml). Flavonoids are secondary metabolites which have been found to have antioxidant, antibacterial and antimicrobial properties (Halliwell & Gutteridge, 1995; Pandey et al., 2018). It was observed that during storage the flavonoid contents of the juices decreased gradually as the storage period increased, this was obviously noticed at four months of storage. Phytochemical phenolic and flavonoid compounds are easily deteriorated under storage, this is due to their susceptibility to various conditions of temperature, light, pH, and oxygen, resulting in loss of their bioavailability and antioxidant activities (Giuffrè et al., 2019). Interestingly, reduction in flavonoids in the mixed fruit samples might be as a result of their prolonged storage under ambient condition (28–32 °C). However, the superiority of the flavonoid contents of bitter orange juice over pineapple juice was remarkably noticed along the mixing ratio, where samples that contained higher percentage of bitter orange juice had higher flavonoid content compared with the samples that contained lower percentage of bitter orange juice.

3.3.2. Total phenolic content

The phenolic content of the mixed fruit juices decreased from 0.28 to 0.10 mg/ml as influenced by the mixing ratio (Table 4). Freshly prepared 100% bitter orange juice sample recorded the highest value, while 100% pineapple juice sample that had been stored for 6 months had the lowest value (0.10 mg/ml). This indicate that bitter orange is a good complementary source of phenolics, a compound associated with free radicals mopping up through hydrogen or electron-donating as well as metal ion chelating agents (Arueya et al., 2017; Rice-Evans & Bourdon, 1993). Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee et al., 2005). The decrease in the total phenolic contents of all the samples were minimal throughout the storage period. Slight reduction in total phenolic contents during storage might be due to phenolic compounds' fragile structures and their susceptibility to environmental conditions such as light, oxygen and temperature (Kim et al., 2018). Antioxidant properties of each fruit juice confer on it the potential as a better antimicrobial agent than the conventional antibiotic. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity and since most fruits were considered as a potent source of phenolic compounds and most importantly it plays a vital role in the prevention of many diseases (Aadil et al., 2015).

3.3.3. Total antioxidant

The total antioxidant content of the mixed fruit juice declined from 1.01 to 0.25 mg/ml (Table 4). Highest value was recorded in 100% fresh pineapple juice, while freshly prepared 100% bitter orange had the lowest value. Decrease in total antioxidant contents of the juice samples was observed during storage, and this progressed as the storage period increased. The reduction in total antioxidants might be due to their sensitivity and long term exposure to ambient storage temperature. This findings is in accordance with the results of Mohamad Salin, Md Saad, Abdul Razak, and Salim (2022) who reported on the influence of storage temperature on antioxidant activities of water melon juice and Shourove et al. (2020) who worked on effect of thermal treatment on the antioxidant properties of star fruit juice. Sample with 100% pineapple had the highest value followed by sample having 25% bitter orange and 75% pineapple juice. Their richness in total antioxidant capacity, suggest that they might also possess good radicals scavenging activities or ability to inhibit free radicals, which are causing degenerative diseases (Sin et al., 2013; Willis et al., 2009). Surprisingly, 100% pineapple juice had a higher total antioxidant capacity than 100% bitter orange juice, despite the higher contents of phenolics, flavonoids, carotenoids, DPPH radical scavenging activity and reducing power of bitter orange juice. This might be as result of other compounds in pineapple such as vitamin E and bromelain that possess antioxidant properties.

3.3.4. Total carotenoid

The carotenoid values of the mixed fruit juices ranged from 0.03 to 0.98 mg/ml (Table 4). It was observed that freshly prepared 100% bitter orange juice sample possessed the highest value, while 100% pineapple juice sample that had been stored for 6 months had the lowest value (0.03 mg/ml). Carotenoids are a group of phytochemicals that are responsible for the yellow, orange and red colors of some fruits (Wang et al., 2008). They are also precursor of vitamin A (Zulueta et al., 2007). Oranges are found to be among the fruits which are rich in carotenoid (Zulueta et al., 2007). However, the carotenoids stability during ambient storage is very crucial for the attractiveness and acceptability of the fruit juice products (Baker & Günther, 2004). Interestingly, the carotenoid contents of the juices samples decreased maximally after five months of storage. The reduction in the carotenoid contents might be as a result of its oxidation and isomerization during storage which eventually affected the juice samples colour (Baker & Günther, 2004).

3.3.5. DPPH(1,1-diphenyl-2 picrylhydrazyl)

The DPPH values of the mixed fruit juices reduced from 91.28 to 14.95% (Table 4). Highest value was recorded in the freshly prepared sample that contained 75% bitter orange and 25% pineapple, 100% pineapple juice sample that had been stored for 6 months had the lowest value. During storage period, maximum decrease in DPPH was observed in the samples. The decrease in DPPH of the samples might be due to the ambient storage temperature and longer period of storage. Similar result was reported by Mohamad Salin et al. (2022) who researched on effect of storage temperature on the physico-chemical and antioxidant properties of watermelon juice (*Citrullus lanatus*) and observed a great reduction in DPPH value of water melon juice stored at room temperature. The DPPH assay measures the hydrogen-donating antioxidant (AH) activity of tested substances. DPPH is stable radical, which in its radical form gives a violet color (Molyneux, 2004). It was noticed that as the percentage of bitter orange in the mixed fruits increased, the DPPH increased. High DPPH activity observed in almost all the samples indicated that the sample might possess ability to effectively neutralize proton donating compounds that have proton donating ability and could serve as inhibitor of free radical or possibly act as scavenger (Arueya et al., 2017).

3.3.6. Reducing power

The reducing power values of the mixed fruit juice samples decreased from 1.64 to 0.11 mg/ml (Table 4). Freshly prepared 100% bitter orange had the highest value, while 100% pineapple juice sample that had been stored for 6 months had the lowest value. The present study illustrated that the reducing power values of the juice samples decreased over the storage period. Decrease in the reducing power values of the samples over the storage time gave a decrease in the absorbance values, with the fresh 100% bitter orange having the highest

Table 5
Bacterial examination of the mixed fruit juice.

Months	100J (cfu/ml) × 10^2	100P (cfu/ml) × 10^2	75J/25P (cfu/ml) × 10^2	50J/50P (cfu/ml) × 10^2	25J/75P (cfu/ml) × 10^2
0	0.00 ± 0.00 ^{c}	0.20 ± 0.05^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{a}	0.00 ± 0.00^{f}
1	0.00 ± 0.00 ^{c}	1.60 ± 0.03^{f}	0.00 ± 0.00^{f}	0.05 ± 0.01^{f}	0.10 ± 0.02^{e}
2	0.00 ± 0.00 ^{c}	1.80 ± 0.06^{e}	0.10 ± 0.00^{e}	0.10 ± 0.02^{e}	0.20 ± 0.03^{d}
3	0.01 ± 0.01^{bc}	2.00 ± 0.08^{d}	0.50 ± 0.01^{d}	0.65 ± 0.01^{d}	0.40 ± 0.05^{c}
4	0.02 ± 0.01^{ab}	2.57 ± 0.10^{c}	0.79 ± 0.01^{c}	1.00 ± 0.02^{c}	0.60 ± 0.03^{b}
5	0.02 ± 0.01^{ab}	6.89 ± 0.11^{b}	0.85 ± 0.03^{b}	1.15 ± 0.01^{b}	0.80 ± 0.01^{a}
6	0.03 ± 0.01^{a}	10.20 ± 0.15^{a}	0.95 ± 0.02^{a}	1.20 ± 0.03^{a}	0.81 ± 0.05^{a}

Table 6
Fungal examination of the mixed fruit juice.

Months	100J (cfu/ml) × 10^2	100P (cfu/ml) × 10^2	75J/25P (cfu/ml) × 10^2	50J/50P (cfu/ml) × 10^2	25J/75P (cfu/ml) × 10^2
0	0.00 ± 0.00^{c}	0.30 ± 0.02^{g}	0.00 ± 0.00^{f}	0.10 ± 0.01^{g}	0.00 ± 0.00^{f}
1	0.00 ± 0.00^{c}	0.50 ± 0.01^{f}	0.10 ± 0.01^{f}	0.10 ± 0.02^{f}	0.20 ± 0.01^{e}
2	0.00 ± 0.00^{c}	0.50 ± 0.02^{e}	0.10 ± 0.02^{e}	0.20 ± 0.02^{b}	0.20 ± 0.03^{d}
3	0.00 ± 0.00^{bc}	0.70 ± 0.02^{d}	0.20 ± 0.03^{d}	0.30 ± 0.03^{d}	0.30 ± 0.02^{c}
4	0.00 ± 0.00^{ab}	0.80 ± 0.01^{c}	0.30 ± 0.01^{c}	0.40 ± 0.04^{c}	0.50 ± 0.02^{b}
5	0.00 ± 0.00^{ab}	0.83 ± 0.05^{b}	0.35 ± 0.03^{b}	0.45 ± 0.01^{b}	0.55 ± 0.02^{a}
6	0.00 ± 0.00^{a}	0.95 ± 0.04^{a}	0.40 ± 0.02^{a}	0.55 ± 0.03^{a}	0.60 ± 0.04^{a}

ferric reducing power (1.64 mg/ml). Reducing power of a substance is a reflection of its ability to release electrons to facilitate reduction of ferric ions Fe3+ to ferrous ions Fe2+ (Rice-Evans et al., 1997; Arueya et al., 2017).

3.4. Microbial analyses of mixed fruit juice under storage

The results of microbial analysis of the different ratio mixed juice samples are presented in Tables 5–6. The bacteria counts varied from 0 to 10.20 × 10^2 cfu/ml for bacteria, while the fungi counts ranged from 0 to 0.95 × 10^2 cfu/ml. The results obtained for total bacteria counts and total fungi counts of the freshly produced pasteurized fruit juice samples showed that no growth was recorded in all the mixed fresh juice samples, except for the 100% fresh pineapple juice sample, where a count of 0.20 × 10^2 cfu/ml for bacteria and 0.30 × 10^2 cfu/ml for fungi were reported. There were significant differences in both fungi and bacteria population at 95% confidence level. The acid and sugar ratio have significant influence on the survival of microbes and also greatly affect the storage stability and sensory quality of the juice samples (Jay, 2000; Ezeama, 2007). It could be deduced that both the bacteria and fungi counts decreased as the percentage inclusion of bitter orange juice increased and this also reduced their proliferation during storage. This indicate the possibility of the bitter orange juice to serve as a natural preservative to other fruit juice samples, which is a pointer to good manufacturing and handling practice (Ezeama et al., 2007). The recommended microbial limit for commercial fruit juices as specified by SON (2008, pp. 1–16) is 2.0 × 10^2 CFU/100 ml, while the recommended limit for microbes in food is 1.0 × 105 cfu/g (Priyadarshini et al., 2012). Note-worthily, the microbial contents (bacteria and fungi) of the juice containing bitter orange were below the limit after six months of storage, indicating that juice containing bitter can be kept in good quality beyond six months of storage. Contrarily, 100% pineapple juice sample experienced high microbial proliferation beyond the limit for commercial juice after three months of storage, implying that its safety cannot be guaranteed after three months of storage.

4. Conclusion

The present study indicates that a good quality value added product could be produced from bitter orange and pineapple juice. The product quality was influenced by the level of inclusion of bitter orange juice in pine apple juice and the length of storage. The product quality was acceptable up to 4 months, indicating that it could be stored for 120 days without any preservatives at room temperature. Juice sample having 25% of bitter orange and 75% pineapple was the most preferred by panelists, possessing high antioxidants capacities which can help in fighting degenerative diseases and better keeping quality.

Author's statement

AKEEM OLAYEMI RAJI: He is the pricipal investigator, and was involved in the design concept, data acquisition, analysis, interpretation and manuscript drafting. His contribution to the work is rated 40%

OLUBUMI FLORENCE ADEBAYO: She is a co-investigator, and was involved in the data acquisition, analysis, interpretation and manuscript drafting. Her contribution to the work is rated 31%

SAHEED MAYOWA SANUSI: He is a co-investigator, and was involved in the design concept, data analysis, interpretation and manuscript drafting. His contribution to the work is rated 29%

Declaration

The authors declared that there is no conflict of interest on this article.

Data availability

Data will be made available on request.

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