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Interaction between orange juice and < 1 kDa leaf peptides: effect on the antioxidant and antidiabetic related enzyme inhibitory activities

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Abstract

Designing a good vehicle for functional ingredients is the major focus of this study. Small molecular weight peptides (< 1 kDa) extracted from amaranth leaf protein (ALP), eggplant leaf protein (ELP) and fluted pumpkin leaf protein (FLP) were incorporated into freshly prepared orange juice at an effective and inhibitory concentrations of the peptides. The rate of degradation of ascorbic acid was more in the control juice (140.06 to 18.43 mg/mL) when compared with juice containing peptides at both storage conditions (140.08 to 32.32 mg/mL). However, the rate of ascorbic acid reduction during storage (refrigerated and ambient) was least in the juice containing peptide, isolated from ELP when compared with the juice samples that contained peptides isolated from ALP and FLP. After the eighth week of storage, juice that contained FLP peptide had greater amounts of residual total phenolic content (370.53 & 432.33 µg GAE/100 mL), juice that contained ALP peptide retained better ability to scavenge DPPH radicals (52.32 & 66.84%) while juice sample that contained ELP retained more metal chelating activities (44.82 and 51.03%). The results of antidiabetic property showed that juice containing peptide isolated from ALP contained greater amounts of α-amylase inhibitory activity (41.50 and 46.89%) while greater amounts of α-glucosidase inhibitory activities were retained in juice that contained peptide isolated from FLP. The results concluded that orange juice may be considered a veritable vehicle for functional ingredients for improved health.

Keywords Food vehicle, Functional drink, Functional ingredients, Bioactive compounds

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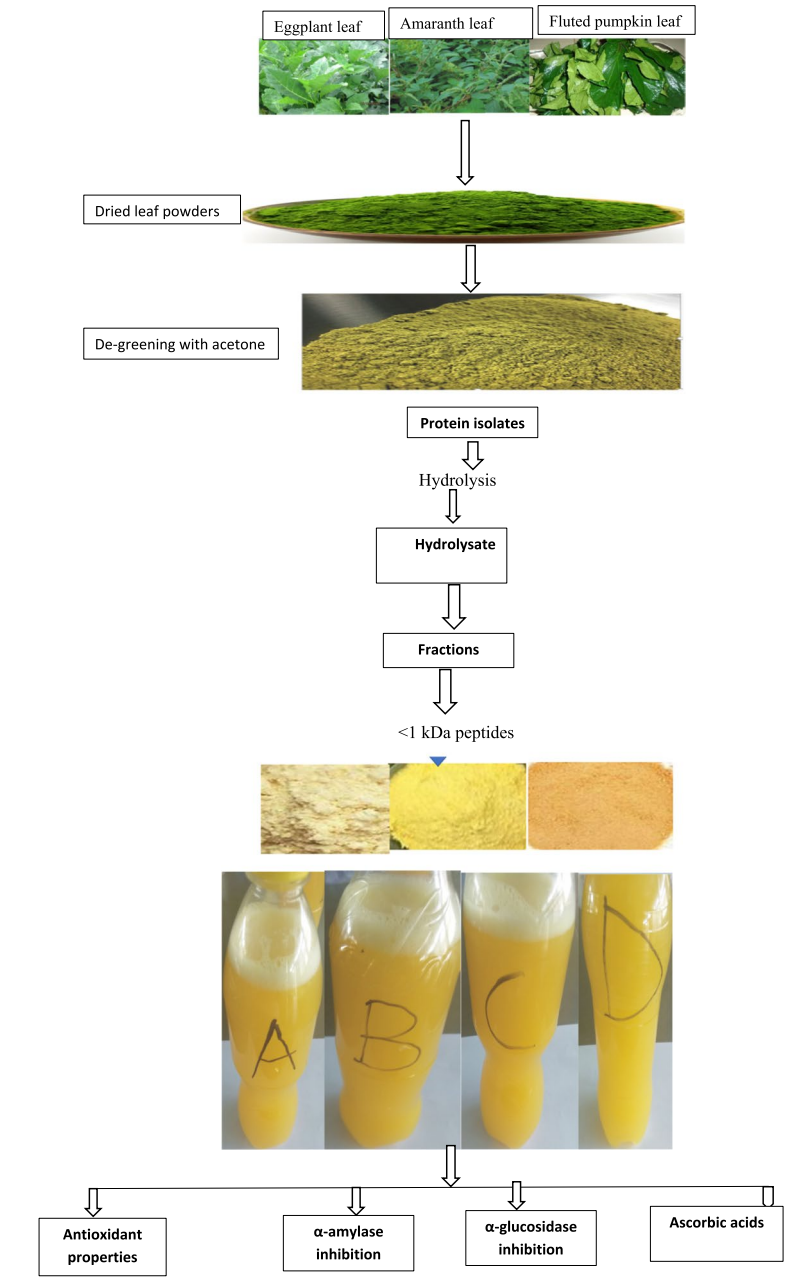
Graphical Abstract



Note: A=Amaranth leaf peptides containing juice
B=Eggplant leaf peptides containing juice
C=Fluted pumpkin leaf peptides containing juice
D=Control without peptides

Introduction

Beverages have been recognized as an excellent vehicle to deliver nutrients and bioactive compounds, such as vitamins, minerals, antioxidants, ω-3 fatty acids, plant extracts, fiber, prebiotics, and probiotics. Such class of beverage, that contained added ingredients are called functional beverages (Otles & Cagindi 2012). Due to the perceived health benefits of functional beverages and the trend of people towards unhealthy lifestyle, leading to various diseases such ashypertension and diabetics, the market of functional beverages has become one of the fastest growing markets in the food sector (Anushere et al., 2023).

Orange (*Citrus sinensis* L. Osbeck) juice is the liquid that is naturally contained in orange fruit, which may be prepared by mechanically squeezing or macerating fruit flesh (Hollis et al. 2009). Orange juice has been suggested to be a health promoting beverage, due to the presence of antioxidant tand many bioactive compounds like ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidine, vitamin-C, carotenoid and naringin (Xu et al. 2008). However, ascorbic acid, which is a major antioxidant in the juice is unstable, and its deterioration influence the overall nutritional quality ofthe juice (Lanny & Lie 2014). The rate of ascorbic acid degradation increases during storage, depending on the conditions in the stored environment (Burdulu et al. 2006). Differences in the storage temperatures (refrigeration and ambient) is one of the factors that influence the quality characteristics of juice, and may have greater influence on its shelf life (Zhang et al. 2016). The shelf stability studies of orange juice have been widely studied under different storage conditions by various authors (Lanny & Lie 2014; Zhang et al. 2016). Studies have suggested that the biochemical changes of orange juice that adversely affect its shelf life may be minimized through the addition of synthetic preservatives, such as sodium benzoate (Xu et al. 2008; Marwa 2018). Natural or plants extracts, perceived to contain antioxidant or preservative effect have also been suggested to enhance the bioactivity or extend the shelf life of juice (Marwa 2018). Such plant extracts or plant derived bioactive ingredients may have been added to the juice as a way of finding appropriate vehicle for the functional ingredients to deliver the perceived bioactive functions to the body or strengthen the bioactive functions of the enriched juice. In either way, the plant extracts may systematically serve as preservative to the juice.

Bioactive peptides are specific protein fragments with positive impact on the health of the body. The bioactivities of peptides are determined by the amino acid composition and sequence, once they are released from the encrypted parent protein (Aluko 2015). Leafy vegetables contain appreciable amounts of proteins from which peptides can be obtained (Famuwagun et al. 2020a). Peptides fractions that demonstrated potentials antioxidant, antidiabetic and antihypertensive properties have been isolated from some edible leaves in Nigeria, such as fluted pumpkin (Famuwagun et al. 2020b), African eggplant (Famuwagun et al. 2021) and amaranth leaves (Famuwagun et al. 2020c).

Orange juice has been used as vehicle for a number of plants derived functional ingredients, such as water mint leave extract (Marwa 2018), probiotics (Horáčkováet al. 2018), prebiotic beads in Thailand (Wunwisa & Kamolnate 2010), rice bran derived peptide (Amanda et al. 2016), Fructooligosaccharides (Renuka et al. 2009), rice bran (Amanda et al. 2016), Kersting'sgroundnut proteins (Osungbade et al. 2021),which suggest fruit juices may be good transport media of functional ingredients. However, there is a dearth of information in the literature on the use of orange juice as a vehicle for leaf derived peptides as well as the associated interactions. This study examined the possibility of orange juice as a potential vehicle for peptides obtained from enzymatic hydrolysates of amaranth, African eggplant and fluted pumpkin leafy vegetables through the effects on the physicochemical properties, ascorbic acid contents, antioxidant, α-amylase, α-glucosidase enzyme inhibitory activities as well as the stability of the peptides in the juice, both in the ambient and refrigerated conditions.

Materials and methods

Materials

Enzymes (rat intestinal acetone powder as source of α-glucosidase, porcine pancreatic α-amylase, and other reagents (4-nitrophenyl α-D-glucopyranoside), Folin Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) free radical powder, Ferrozine powder and $FeCl_2$, Folin reagent used in this study were of analytical grade and purchased from accredited representative of Sigma (St. Louis, MO, USA), The ultrafiltration membranes (1weight cut-offs, MWCO) were purchased from Fisher Scientific (Oakville, ON, Canada). Ascorbic acid, 2,6-Dichloroindophenol (DCIP), are of analytical grade supplied by Sigma (Madrid, Spain). Fresh leaves of amaranth, eggplant, fluted pumpkin and Ripe oranges (*citrus sinensis*) were obtained from an accredited vegetable farm in Osun State, Nigeria.

Preparation of leaf powders, proteins isolates and hydrolysates

The fresh leaves of amaranth, eggplant and fluted pumpkin were sorted, de-stalked, rinsed with water, and dried using a hot air oven (Uniscope SM9053 Laboratory Oven, Singerfried, England) at $55 \pm 2^{\circ}C$ for 8 h. The dried leaves were milled to powder using a laboratory blender (VLC

sapphire grinder, IS-4930, England). Chlorophylls and the other polyphenolic compounds were removed by mixing the leaf powders with acetone at a ratio of 1:10 (leaf power to acetone), stirred continuously for 2 h, and then filtered using a muslin cloth. The filtered residue was subjected to a second round of acetone extraction and the final residue obtained was air-dried in a fume hood at room temperature for 48 h, packaged as the leaf powder and stored at -20°C (Famuwagun et al. 2020a).

Proteins were isolated from the packaged leaf powder using the method described by Malomo et al. (2014) which was slightly modified by Famuwagun et al. (2020a)using the combined processes of solubilization, precipitation and neutralization.The protein isolates obtained above were hydrolysed using different proteases (purchased from Sigma-Aldrich, St. Louis, MO, USA), namely Alcalase (> 2.4 U/g), chymotrypsin (> 250 units/mg),pepsin (> 250 units/mg), at an enzyme: substrate ratio of 1:100. Different hydrolysis conditions were employed as follows: Alcalase (pH 8.0, 50 °C);pepsin (pH 2.0, 37 °C), and chymotrypsin (pH 8.0, 37 °C). The pH of the hydrolysis process was maintained using either 1 M NaOH or 1 M HCl as appropriate, while temperature was kept constant with a thermostat. After the 4-h hydrolysis, the enzymes were inactivated by heating and holding at 85 °C for 15 min followed by centrifugation (9000×*g*, 4 °C, 30 min), and the supernatant was freeze-dried to obtain the respective enzymatic protein hydrolysate and stored at -20 °C.

Membrane ultra-filtration fractionation of less than 1 kDa peptide fractions

Preparations of pepsin hydrolysate (PH), chymotrypsin hydrolysate (CH) and alcalase hydrolysate (AH) from amaranth, eggplant and fluted pumpkin leaves as well as the ultrafiltration process of < 1 kDa peptide fractions and its bioactivities have been reported in our previous studies (Famuwagun et al. 2020a).

Preparation of orange juice and the fortified orange juice samples

Sample collection and preparation

Matured and ripe sweet oranges (*Citrus sinensis*), three days after harvest were purchased from the Teaching and Research Farms, Obafemi Awolowo University, Ile-Ife, Nigeria. The fruits were sorted and defect free oranges were selected for the juice extraction. The selected fruits were sorted and washed thoroughly under running tap water. The pericarps were removed with the aid of stainless-steel knife and the juice was extracted using a hand-held juice extractor to obtain a clear orange juice. The fresh orange juice produced was distributed equally into 162 different sterilized polyethylene bottles. The peptides were incorporated into the fresh juice (Fig. 1) in the container in a single concentration: 0.190 mg/mL, 0.185 mg/mL and 0.193 mg/mL for peptides derived from amaranth (ALP), eggplant (ELP) and fluted pumpkin (FLP) leaves respectively. These translated into 19.0, 18.5, 19.3 mg per 100 mL serving of the enriched orange juice beverage. The peptide levels were determined based on the minimum average concentrations that inhibited at least 50% enzymes (α-amylase and glucosidase) concentration and scavenged 50% free radicals (DPPH, hydroxyl and superoxide radicals) as reported by Famuwagun, et al.(2020b); Famuwagun et al. 2020c), (2021) and the mixture was homogenized before aliquots were taken for analysis.

For shelf stability, peptide incorporated juice samples and the control juice (juice without peptide) were capped and pasteurized at 80° C for 10 min. The juice samples were cooled under running water and stored at ambient (28 ± 2° C) or refrigerated (4 ± 2° C) conditions for eight weeks. Every week, representative of each sample was taken out and evaluated for changes in the ascorbic acid content, total phenolic content, antioxidant properties (DPPH scavenging activities and metal chelating activities) and enzyme inhibitory activities (alpha amylase and glucosidase inhibition).

Estimation of ascorbic acid content

Ascorbic acid content was estimated by titrimetric method described by Rekha et al. (2012). Five milliliters of standard ascorbic acid (100 µg/mL) were measured into a conical flask containing 10 mL 4% oxalic acid. The mixture was titrated against the 0.0005 M of 2, 6-dichlorophenol indophenols dye (DCPIP) (prepared by dissolving 145 mg DCPIP in 100 mL hot distilled water and a subsequent addition of 300 mL of 0.066 M phosphate buffer, pH = 6.98, previously prepared by mixing the respective volumes of sodium dihydrogen phosphate and sodium mono-hydrogen phosphate solutions (2/3 ratio) and distilled water was added to the final volume of 1000 mL). The appearance and persistence of pink colour for 15 s was taken as the end point. The amount of dye consumed (V1 mL) is equivalent to the amount of ascorbic acid. Five milliliters of diluted juice sample (prepared by taking 5 mL of juice in 100 mL 4% oxalic acid) was measured inside a conical flask containing 10 mL 4% oxalic acid in a conical flask and titrated against the dye (V2 mL). The amount of ascorbic acid was calculated using the formula;

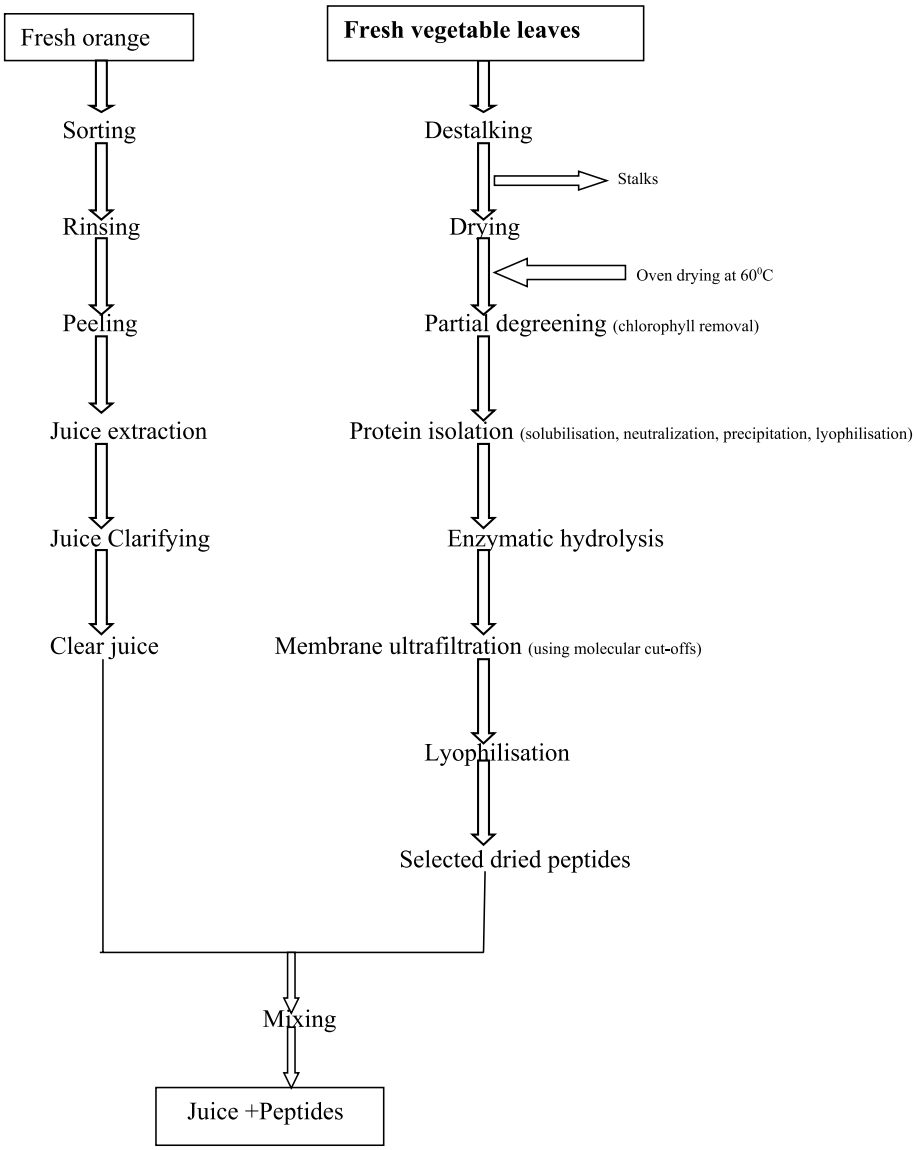


Fig. 1 Process flowchart for the preparation of orange juice containing peptides. OJ+ALP: Orange juice incorporated with peptide obtained from Amaranth leafy vegetable; OJ+ELP: Orange juice incorporated with peptide obtained from eggplant leafy vegetable; OJ+FLP: Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable; Control: Orange juice only; Amb.: Ambient condition; Ref.: Refrigerated condition

$$Ascorbic\ acid\ (mg/mL) = \frac{Xi\ mg}{V1} x \frac{V2}{15\ ml} x \frac{100\ mL}{mL\ of\ sample\ used\ for\ analysis} \quad (1)$$

$Xi(mg) = quantity\ of\ ascorbic\ acid\ dissolved\ in\ a\ known\ volume\ of\ oxalic\ acid V1 = volume\ of\ dye\ consumed\ by\ the\ standard$

$$V2 = volume\ of\ dye\ consumed\ by\ the\ sample$$

$$15\ mL = total\ volume\ of\ sample\ and\ oxalic\ titrated$$

$$100\ mL = volume\ of\ oxalic\ acid\ solution\ used\ in\ dissolving\ the\ sample$$

Determination of total phenol content

The total phenolic content (TPC) was determined following the method described by Gulcin et al. (2006) using the Folin Ciocalteu's phenol reagent which is an oxidizing reagent. A 10-fold dilution of Folin–Ciocalteu reagent was prepared just prior to use. To 100 µL of the juice was added 900 µL of distilled water to give 10-folds dilution. Two hundred microliters (200 µL) of freshly prepared diluted Folin-Ciocalteu's phenol reagent was added and the mixture was vortexed. After allowing the mixture to equilibrate for 5 min, the reaction was then neutralized with 1.0 mL of 7% (*w/v*) Na_2CO_3 solution. After 2 h of incubation at room temperature, the absorbance was measured at 750 nm. A standard curve was prepared with a linear range of 0.0–0.1 mg/mL using Gallic acid. The results were expressed as milligram Gallic acid equivalent (µgGAE/ ml) of juice and this was obtained by extrapolation from the standard curve. Distilled water was used as blank.

Antioxidant activities

1, 1- Diphenyl picrylhydrazine (DPPH) radical scavenging activity

DPPH free radical scavenging activity was determined using the modified method described by Rheka et al. (2012). DPPH was dissolved in 95% methanol to a final concentration of 100 µM. The sample was diluted five times with distilled water and 1mL aliquot of each sample was mixed with 1 ml of the 100 µM DPPH radical solution in a clean test-tube and incubated in the dark for 30 min. Distilled water was used in the blank assay while ascorbic acid powder served as the positive control. Absorbance was measured at 517 nm using a spectrophotometer and the percentage DPPH radical scavenging activity was determined using the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(Ab - As)}{Ab} x100 \quad (2)$$

where Ab and As, are absorbance of the blank and sample respectively for DPPH radical scavenging activity.

Metal chelation activity

The metal (iron) chelating activity (MCA) of the was determined according to the modified method of Xie et al. (2008). A 1 mL aliquot of the sample or blank (distilled water) was mixed with 50 µL of 2 mM $FeCl_2$ and 1.85 mL distilled water in a reaction tube. This was followed by the addition of 100 µL of 5 mM Ferrozine. The mixture was vortexed thoroughly and incubated at room temperature for 10 min and absorbance values of both the blank (Ab) and samples (As) were measured at 562 nm using spectrophotometer. The metal chelating activity was calculated as follows;

$$Metal\ chelating\ activity\ (\%) = \frac{(Ab - As)}{Ab} x100 \quad (3)$$

where Ab is the absorbance of the blank and As is the absorbance of the samples for metal chelating activities.

Enzyme inhibitory activities

Inhibition of α-amylase enzyme

Inhibition of porcine α-amylase activity was determined using the dinitrosalicylic acid described by Kwon et al. (2006). One hundred microlitres (100µL) of diluted juice (1:10) prepared in 0.02 M phosphate buffer, pH 6.9 containing 0.006 M NaCl was added to 100µL of α-amylase (1 mg/mL final concentration in0.02 M phosphate buffer, pH 6.9 containing 0.006 M NaCl). The mixtures were pre-incubated at 28°C for 10 min and 200 µL of 1% starch (prepared in 0.02 M phosphate buffer, pH 6.9 containing 0.006 M NaCl) was added. The reaction mixtures were incubated at room temperature for 10 min. The reactions were stopped by incubating the mixture in a boiling water bath for 5 min after adding 1 mL of dinitrosalicylic acid. The reaction mixtures were cooled to room temperature, diluted to 1:5 ratio with distilled water and the absorbance was measured in a at 540 nm using spectrophotometer. The percentage inhibition of enzyme activity was calculated as.

$$Alpha{-}amylase\ inhibition = \frac{(Absorbance\ of\ control - (Absorbance\ of\ sample - Absorbance\ of\ sampleblank))}{Absorbance\ of\ control} x100 \quad (4)$$

Inhibition of α-glucosidase enzyme

Inhibition of α-glucosidase activity was determined using yeast α-glucosidase and p-nitrophenyl- α-D-glucopyranoside (pNPG) as described by Kim et al. (2004). One hundred microlitres (100μL) of diluted juice sample (five-fold dilutions) or standard (acarbose; 0.25 mg/mL final concentrations dissolved in 0.1 M phosphate buffer, pH 6.9) was added to 50μL of α-glucosidase (1 mg/mL final concentration in0.1 M phosphate buffer). The mixtures were pre-incubated at 37°C for 20 min. After pre-incubation, 100 μL of 5mM pNPG (dissolved in 0.1 M phosphate buffer, pH 6.9) was added and incubated at 37°C for 10 min and the absorbance was read at 405 nm using spectrophotometer. Percentage of inhibition of the enzyme activity was calculated as;

$$\alpha-glucosidase\,inhibition = \frac{(final\ absorbance\ of\ blank - (final\ absorbance\ of\ sample)}{final\ absorbance\ of\ blank} x100 \quad (5)$$

Statistical analysis

Analyzes were carried out in triplicates and data presented as means ± standard deviation. The two-way analysis of variance post hoc test was carried out with the Duncan's Multiple Range test at ($p < 0.05$) using a Statistical Package for Social Science (SPSS, Version 22) software.

Results and discussion

Ascorbic acid

Vitamin C, also known as ascorbic acid is an important component of fruit juice. The quantity of vitamin C in fruits juices are used as quality index of the juice. The vitamin C content of the juice samples ranged between 140.06 and 140.08 mg/mL. The peptide is not expected to contribute significantly to the vitamin C content of the resulting juice samples at the initial stage. Sridevi et al. (2018) reported non-significant difference in the vitamin C contents of control juice and juice fortified with plant sterol at the day zero. In another study, Marwa (2018) reported 78.57 mg/mL for vitamin C content of

Table 1 Ascorbic content (mg/ 100 ml) of the stored samples

Ambient storage				
Storage weeks	OJ + ALP	OJ + ELP	OJ + FLP	Control
Baseline	$140.08 \pm 1.03^{a}{}_{a}$	$140.07 \pm 1.25^{a}{}_{a}$	$140.07 \pm 1.24^{a}{}_{a}$	$140.07 \pm 1.21^{a}{}_{a}$
1	$130.51 \pm 2.03^{b}{}_{b}$	$138.09 \pm 1.33^{b}{}_{a}$	$80.33 \pm 0.94^{b}{}_{c}$	$60.94 \pm 0.95^{b}{}_{d}$
2	$70.96 \pm 0.22^{c}{}_{b}$	$91.47 \pm 0.94^{c}{}_{a}$	$50.04 \pm 1.22^{c}{}_{c}$	$40.64 \pm 0.44^{c}{}_{d}$
3	$52.01 \pm 0.21^{d}{}_{b}$	$91.17 \pm 0.44^{d}{}_{a}$	$46.94 \pm 1.03^{d}{}_{c}$	$35.43 \pm 0.92^{d}{}_{d}$
4	$50.04 \pm 0.55^{e}{}_{b}$	$89.17 \pm 1.32^{e}{}_{a}$	$43.95 \pm 1.23^{e}{}_{c}$	$28.83 \pm 0.44^{e}{}_{d}$
5	$46.01 \pm 0.45^{f}{}_{b}$	$70.13 \pm 1.33^{f}{}_{a}$	$40.54 \pm 0.95^{f}{}_{c}$	$18.93 \pm 1.03^{f}{}_{d}$
6	$45.05 \pm 0.44^{g}{}_{b}$	$70.08 \pm 0.93^{f}{}_{a}$	$40.34 \pm 1.33^{g}{}_{c}$	$18.53 \pm 1.43^{g}{}_{d}$
7	$32.22 \pm 0.51^{h}{}_{c}$	$70.00 \pm 2.54^{g}{}_{a}$	$40.15 \pm 0.56^{h}{}_{b}$	$18.13 \pm 0.94^{h}{}_{d}$
8	$32.02 \pm 1.04^{i}{}_{c}$	$50.78 \pm 1.33^{h}{}_{a}$	$40.04 \pm 1.22^{i}{}_{b}$	$17.43 \pm 1.03^{i}{}_{d}$
Refrigerated storage				
Storage weeks	OJ + ALP	OJ + ELP	OJ + FLP	Control
Baseline	$140.08 \pm 1.23^{a}{}_{a}$	$140.07 \pm 1.21^{a}{}_{a}$	$140.07 \pm 1.13^{a}{}_{a}$	$140.07 \pm 0.65^{a}{}_{a}$
1	$140.00 \pm 1.43^{a}{}_{a}$	$139.67 \pm 0.56^{b}{}_{b}$	$110.85 \pm 0.55^{b}{}_{c}$	$80.33 \pm 1.23^{b}{}_{d}$
2	$135.58 \pm 0.65^{b}{}_{a}$	$121.63 \pm 1.33^{c}{}_{b}$	$60.94 \pm 1.21^{c}{}_{c}$	$60.94 \pm 0.65^{c}{}_{d}$
3	$133.33 \pm 0.66^{c}{}_{a}$	$111.83 \pm 0.65^{d}{}_{b}$	$60.44 \pm 0.65^{d}{}_{c}$	$42.41 \pm 0.96^{d}{}_{d}$
4	$70.47 \pm 0.32^{d}{}_{b}$	$111.03 \pm 0.67^{e}{}_{a}$	$57.24 \pm 0.66^{e}{}_{c}$	$32.91 \pm 1.15^{e}{}_{d}$
5	$51.23 \pm 0.66^{e}{}_{c}$	$110.33 \pm 0.65^{f}{}_{a}$	$53.94 \pm 0.94^{f}{}_{b}$	$22.49 \pm 0.06^{f}{}_{d}$
6	$50.37 \pm 1.04^{f}{}_{c}$	$91.14 \pm 1.04^{g}{}_{a}$	$51.34 \pm 1.45^{g}{}_{b}$	$22.41 \pm 0.62^{g}{}_{d}$
7	$50.15 \pm 0.54^{g}{}_{c}$	$80.66 \pm 1.33^{g}{}_{a}$	$50.72 \pm 2.06^{h}{}_{b}$	$21.47 \pm 0.96^{h}{}_{d}$
8	$50.11 \pm 0.99^{g}{}_{b}$	$80.46 \pm 1.04^{h}{}_{a}$	$50.24 \pm 1.95^{i}{}_{c}$	$21.40 \pm 1.04^{i}{}_{d}$

Values are reported as mean ± s.d. of replicate determination. Values along the same column with different alphabet as superscript are significantly different at $p < 0.05$ from one another. Values along the same on the same row with different alphabet as subscript are significantly different at $p < 0.05$ from one another

OJ + ALP Orange juice incorporated with peptide obtained from Amaranth leafy vegetable, *OJ + ELP* Orange juice incorporated with peptide obtained from eggplant leafy vegetable, *OJ + FLP* Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable, *Control* Orange juice only, *Amb* Ambient condition, *Ref* Refrigerated condition

Mean values that contain different alphabets at the superscript across the row are signficantly different ($p<0.05$)

Mean values that contain different alphabets at the subscripts along the column are signficantly different ($p<0.05$)

orange juice. The difference may be explained on the basis of degree of ripeness and stage of harvest of the orange fruits. There were drastic reductions in the vitamin C content of the juice samples as the storage periods increased (Table 1), which may suggest oxidation of ascorbic acid to dehydroascorbic during storage. At the ambient storage (Table 1), the residual vitamin C of the juice samples were 17.43, 32.02, 50.78 and 40.04 mg/100 mL for control and juice fortified with ALP, ELP and FLP respectively. Whereas, the residual amounts of vitamin C at the refrigerated temperature (Table 1) were 21.40, 50.11, 80.46 and 50.24 mg/100 mL, for control juice and juice samples fortified with ALP, ELP and FLP respectively, from the initial amounts of 140.06-140.08 mg/mL and the values were significantly ($p < 0.05$) different from one another. The results showed a decline in the loss of ascorbic acid at the refrigerated condition when compared to ambient storage condition. This may be attributable to a decrease in the amounts of oxygen available to cause the oxidation of ascorbic acid to dehydroascorbic acid at low storage temperature, since the presence of oxygen has been reported to predispose ascorbic acid to aerobic degradation (Singh & Sharma 2017). Similar trends were reported for cashew apple juice (Maia et al. 2001) and red fleshed orange juice (Burdulu et al., 2006). Juice samples fortified with peptides were observed to contain higher vitamin C when compared to the juice without vitamin at both storage temperatures. This pattern may be explained based on the antioxidant history of the added peptides. In this case, the leaf peptides acted as antioxidant and by extension, preservative for the vitamin C in the juice probably by scavenging the oxygen to lower the rate of ascorbic acid oxidation or inhibiting the pathways for the production of dehydroascorbic acid. The results were in line with the report of Marwa (2018) on orange juice mixed with water mint leave and Zheng and Wang (2001) on aloe juice blended Kinnow nectar. Furthermore, the pattern of ascorbic acid retention during storage for the juice samples fortified with different peptides varied. The values of ascorbic acid across the peptides were also significantly ($p < 0.05$) different from one another, with ELP fortified juice having a better retention

Table 2 Total phenolic content (mgGAE/ 100 g) of the stored samples

Ambient temperature				
Storage weeks	OJ + ALP	OJ + ELP	OJ + FLP	Control
Baseline	$844.59 \pm 0.54^{a}{}_{b}$	$844.01 \pm 0.54^{a}{}_{a}$	$844.96 \pm 0.55^{a}{}_{a}$	$840.22 \pm 1.45^{a}{}_{c}$
1	$839.60 \pm 1.23^{b}{}_{b}$	$832.27 \pm 0.33^{b}{}_{c}$	$841.28 \pm 0.53^{b}{}_{a}$	$815.00 \pm 0.54^{b}{}_{d}$
2	$812.67 \pm 1.44^{c}{}_{b}$	$802.56 \pm 0.66^{c}{}_{c}$	$814.29 \pm 0.67^{c}{}_{a}$	$743.35 \pm 1.65^{c}{}_{d}$
3	$739.83 \pm 1.43^{d}{}_{a}$	$718.00 \pm 1.05^{d}{}_{c}$	$738.67 \pm 0.55^{d}{}_{b}$	$644.69 \pm 1.45^{d}{}_{d}$
4	$606.33 \pm 1.33^{e}{}_{c}$	$614.33 \pm 1.13^{e}{}_{b}$	$633.67 \pm 0.51^{e}{}_{a}$	$452.33 \pm 0.54^{e}{}_{d}$
5	$410.81 \pm 1.94^{f}{}_{c}$	$425.32 \pm 0.54^{f}{}_{b}$	$484.65 \pm 1.06^{f}{}_{a}$	$306.71 \pm 0.94^{f}{}_{d}$
6	$394.12 \pm 1.44^{g}{}_{c}$	$422.98 \pm 0.54^{g}{}_{b}$	$471.28 \pm 1.24^{g}{}_{a}$	$281.28 \pm 1.05^{g}{}_{d}$
7	$324.00 \pm 1.84^{h}{}_{c}$	$392.00 \pm 1.05^{h}{}_{b}$	$419.33 \pm 1.04^{h}{}_{a}$	$239.57 \pm 1.22^{h}{}_{d}$
8	$312.48 \pm 0.54^{i}{}_{c}$	$363.33 \pm 1.25^{i}{}_{b}$	$370.53 \pm 1.42^{i}{}_{a}$	$188.65 \pm 1.54^{i}{}_{d}$
Refrigerated temperature				
	OJ + ALP	OJ + ELP	OJ + FLP	Control
Baseline	$844.59 \pm 2.04^{a}{}_{a}$	$844.01 \pm 0.75^{a}{}_{c}$	$843.04 \pm 0.66^{a}{}_{d}$	$840.22 \pm 2.04^{a}{}_{b}$
1	$842.56 \pm 1.54^{b}{}_{a}$	$841.83 \pm 1.05^{b}{}_{b}$	$841.99 \pm 0.42^{b}{}_{c}$	$821.33 \pm 1.85^{b}{}_{d}$
2	$827.62 \pm 0.54^{c}{}_{b}$	$811.33 \pm 1.09^{c}{}_{c}$	$834.00 \pm 0.95^{c}{}_{a}$	$754.25 \pm 1.91^{c}{}_{d}$
3	$786.80 \pm 0.66^{d}{}_{a}$	$753.98 \pm 0.75^{d}{}_{c}$	$768.00 \pm 0.49^{d}{}_{b}$	$685.84 \pm 0.85^{d}{}_{d}$
4	$682.17 \pm 1.05^{e}{}_{c}$	$694.65 \pm 0.65^{e}{}_{b}$	$705.05 \pm 0.59^{e}{}_{a}$	$533.72 \pm 0.99^{e}{}_{d}$
5	$526.83 \pm 1.25^{f}{}_{c}$	$551.98 \pm 0.92^{f}{}_{a}$	$540.09 \pm 0.73^{f}{}_{b}$	$365.98 \pm 1.05^{f}{}_{d}$
6	$380.47 \pm 1.05^{g}{}_{c}$	$486.67 \pm 0.92^{g}{}_{b}$	$501.64 \pm 0.73^{g}{}_{a}$	$323.31 \pm 1.13^{g}{}_{d}$
7	$349.30 \pm 0.75^{h}{}_{c}$	$450.98 \pm 0.58^{h}{}_{b}$	$482.45 \pm 1.05^{h}{}_{a}$	$293.62 \pm 1.05^{h}{}_{d}$
8	$324.33 \pm 0.95^{i}{}_{c}$	$426.67 \pm 0.88^{i}{}_{b}$	$432.48 \pm 1.11^{i}{}_{a}$	$252.54 \pm 1.11^{i}{}_{d}$

Values are reported as mean ± s.d. of replicate determination. Values along the same column with different alphabet as superscript are significantly different at $p < 0.05$ from one another. Values along the same row with different alphabet as subscript are significantly different at $p < 0.05$ from one another

OJ + ALP Orange juice incorporated with peptide obtained from Amaranth leafy vegetable, *OJ + ELP* Orange juice incorporated with peptide obtained from eggplant leafy vegetable, *OJ + FLP* Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable, *Control* Orange juice only, *Amb* Ambient condition, *Ref* Refrigerated condition

Mean values that contain different alphabets at the superscript across the row are signficantly different ($p<0.05$)

Mean values that contain different alphabets at the subscripts along the column are signficantly different ($p<0.05$)

capacity, while FLP had the least ability to retain ascorbic acid, for both ambient and refrigerated storage.This pattern may suggest that the peptides inhibited the ascorbic acid degradation pathways differently, which may be attributed to the different ability of the peptides to retard oxidative reactions as previously reported by Famuwagun et al. (2020b). This is also supported by the fact that the peptides were obtained from different leaves (amaranth vs. eggplant vs. fluted pumpkin leaves) and isolated using different enzymes (pepsin vs. chymotrypsin vs. alcalase). The ascorbic acid retention ability of the peptides in the juice has shown that the peptides are stable in the juice and that the orange juice may be a potential vehicle for the peptides.

Total phenolic content (TPC)

The total phenolic content of juice samples ranged between 840.33 and 844.59 µgGAE/100 mL. and the values obtained were significantly different ($p < 0.05$) from one another (Table 2). The values obtained compared well with 800.40 µg GAE/100 mL and 870.33 µg GAE/100 mL reported for pineapple and guava fruit juices (Khaw et al., 2016) and 805.68 µg GAE/100 mL reported for fermented pomegranate juice (Yuan et al. 2019). Pushkala and Srividya (2014) reported lower (235 µg GAE/100 mL) value for aloe-papaya functional beverage blend. As shown in Table 2, the TPC values of the control juice samples reduced rapidly, making the phenolic content of the samples to be significantly ($p < 0.05$) different from one another, for both ambient and refrigerated storage. The phenolic contents of the peptide incorporated juice samples also reduced, as the storage period progressed, but at a slower rate compared with the control sample. Across the samples, FLP incorporated juice had better phenol retention ability compared with other samples, and the values were significantly ($p < 0.05$) different from one another. All the peptides (ALP, ELP and FLP) incorporated juice samples had greater retention abilities than the control. This was expected because there was no additive to inhibit the reductions of phenols during storage in the control sample. Decrease in the polyphenol content of the juice samples during storage may be attributable to continued interaction of phenols in the juice and some other component of the juice, such as the carotene and sugars during storage. Earlier works on *Morinda citrifolia* L. fruit juice (Malsha et al. 2019) and roselle-fruit juice blends (Mgaya-Kilima et al. 2014) reported decline in the phenolic content as storage time increased. At the end of the 8-week storage periods, the phenolic content of the juice samples was less in the control juice (188.65 & 252.45 µg GAE/100 mL) compared to juice enriched with ALP (315.48 & 324.33 µg GAE/100 mL), ELP (363.33 & 426.66 µg GAE/100 mL) and FLP (370.53 & 432.33 µg GAE/100 mL), from initial values of between 840.33 and 844.59 µg GAE/100 mL. In a similar manner, the phenolic content of juice samples stored at refrigerated condition were higher compared with the ambient stored samples, and this may suggest better retention of phenols at low temperature. Similarly, the results indicated better retention of phenolic contents in juice samples fortified with peptides when compared with control

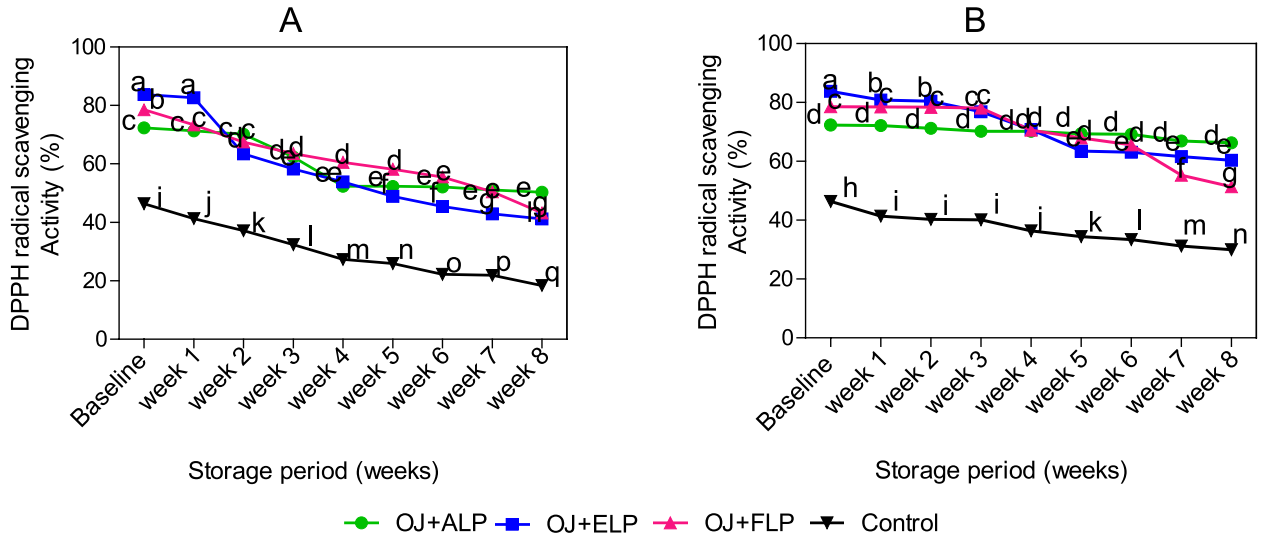


Fig. 2 Graph showing the influence of storage on DPPH radical scavenging activities (%) of samples at Ambient (**A**) and refrigerated storage (**B**) conditions. OJ+ALP: Orange juice incorporated with peptide obtained from Amaranth leafy vegetable; OJ+ELP: Orange juice incorporated with peptide obtained from eggplant leafy vegetable; OJ+FLP: Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable; Control: Orange juice only; Amb.: Ambient condition; Ref.: Refrigerated condition

(although the retention values were significantly different from one anotherat $p < 0.05$) sample and this may suggest strong inhibitory properties of the added peptides, resulting in the reduction of polyphenolic degradation in the juice. Although, the added peptides may not have added phenols to the juice samples, it minimized the rate of reductions of phenols in the juice during storage.

Antioxidant properties

DPPH radical scavenging and metal chelating activities

The DPPH assays were used to indicate the direct activities of samples as primary antioxidant. The DPPH radical scavenging activities of the juice samples ranged between 46.43 and 83.76%. Juice samples fortified with leaf peptides had significantly ($p < 0.05$) higher DPPH radical scavenging activities (72.32–83.37%) when compared with the value (46.83%) obtained for juice without peptide (Fig. 2). Earlier study by Khaw et al. (2016) reported 32.90, 51.40 and 33.70% for DPPH radical scavenging activities of apple, mango and pineapple juices respectively. The DPPH radical scavenging activities of the control sample began to decline after the first week of storage in the ambient and refrigerated condition. However, as shown in Fig. 2, the reduction was slower in the refrigerated condition than the ambient. The reductions in the DPPH radical scavenging activities of OJ + ALP (green line) was not significantly ($p > 0.05$) different in the 1st and 2nd week and later, 4th through 8th week in the ambient storage. The ELP fortified juice (blue line) was stable in the 1st and 2nd week, and then 4th and 5th week. The trend of ELP fortified juice was also similar for FLP + juice (red line) at the ambient condition. Also, the ALP fortified juice was relatively stable throughout the refrigerated storage period, with values of the baseline DPPH radical scavenging activities not significantly different ($p > 0.05$) from the other storage weeks while the ELP and FLP fortified juices were only stable in the last four weeks (4th -8th) of the storage period. In the overall, the result suggested that that low temperature storage is crucial to maintaining the DPPH radical scavenging activities of juice. At the end of the 8th week storage, the residual radical scavenging activities of the control juice (18.41&29.94%) was less compared to the juice fortified ALP (50.32 &66.33%), ELP (41.23 &60.40%) and FLP (43.30 &51.36%) and this may suggest better proton donating ability by the juice containing peptides. The low residual DPPH radical scavenging activity of the juice without peptide may be may be attributable greater loss of ascorbic acid in the juice during storage, since it is the main antioxidant in the juice.

The metal chelating assay was used as an indirect method to measure the activities of the juice samples as secondary antioxidant through the binding of the ferrous (Fe (II)) ion that catalyzes oxidation and subsequently prevents the formation of the Fe (II) − ferrozine complex (Sulaimon and Ooi, 2014). As shown in Fig. 3, At the initial stage, the metal chelating activities of the control juice was 38.59%, juice + ALP (52.10%), juice + ELP (61.03%) and juice + FLP (52.51%). The stronger metal

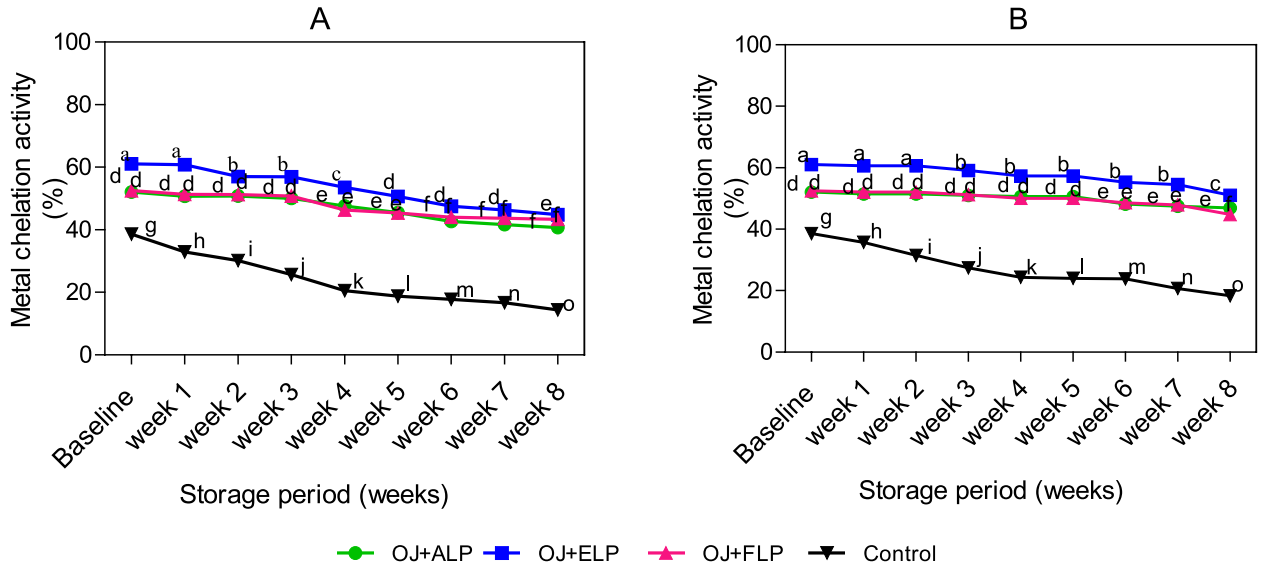


Fig. 3 Graph showing the influence of storage on metal chelating activities (%) of samples at Ambient (**A**) and refrigerated storage (**B**) conditions. OJ+ALP: Orange juice incorporated with peptide obtained from Amaranth leafy vegetable; OJ+ELP: Orange juice incorporated with peptide obtained from eggplant leafy vegetable; OJ+FLP: Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable; Control: Orange juice only; Amb.: Ambient condition; Ref.: Refrigerated condition

chelating activities of the juice fortified with peptides indicate the contribution of the peptides, which is almost twice that initial metal chelating activities of the control juice. For the control juice, the value obtained for metal chelating activity (38.59%) compared well with the values reported for juices obtained from *Garciniaatroviridis* (36.22%), *Myristica fragrans* (28.61%), *Psidium guajava* (44.06%) but higher, when compare with 18.67% reported for juice obtained from *Phyllanthus acidus* fruits (Sulaimon and Ooi, 2014). The metal chelating activities of the juice samples decreased, as the storage period increased, in both storage conditions. The metal chelating activities of the FLP fortified juice (red line) and ALP fortified juice (green line) was stable for the first four weeks of storage (1st -4th) and then in the last three weeks of storage (6th -8th) for the ambient storage condition. The results also showed that the reductions in the metal chelating activities of the ALP and FLP were significantly lower ($p < 0.05$) compared with that of ELP fortified juice (Fig. 2), at both storage conditions.At the 8th week of storage, the metal chelating activities of the control juice had reduced to 14.35 and 18.35%, which were significantly lower ($p < 0.05$) compared to the values obtained in the juice fortified with ALP (40.74 and 46.90%), ELP (44.82 and 51.03%) and FLP (43.33 and 44.75%). The result suggested that the added peptides lowered the rate of decrease of the chelating activities of the fortified juice samples. In a similar manner, the juice samples stored at refrigerated temperature exhibited greater chelating activities, when compared with juice samples stored at ambient conditions, and this may suggest better retention of the chelating abilities of the juice samples at low temperature. Due to dearth of literature information on the metal chelating activities of stored juices, comparison of the values in this study with literature values is limited. The overall results of the antioxidant properties showed that the juice samples may be considered a functional drink, due to improved DPPH free radical and metal chelating activities, when compared with control juice.

Enzyme inhibitory properties

Alpha amylase and glucosidase inhibitory activities

Alpha amylase and glucosidase enzymes play major roles in the digestive systems and are involved in the hydrolysis of starch to glucose and allow the absorption of glucose in the blood streams leading to a rise in the blood glucose levels. Inhibiting these enzymes is one of the effective ways to maintain low blood glucose levels and hence, management of diabetics (Abirami et al. 2014). The alpha amylase inhibitory activities of the control juice sample (42.45%), was significantly lower ($p < 0.05$) compared to the inhibitory activities of the juice samples fortified with ALP (62.83%), ELP (51.77%) and FLP (48.74%) as shown in Fig. 4. The high alpha amylase enzyme inhibition by the fortified juice samples may suggest significant contribution of the added peptides to the activities of the resulting juices. For the inhibitory activities of the control juice (sample without peptide), the value was lower when compared to the value reported for juices obtained from *hystrix* (75%), *maxima* red (82%) and *maxima white*

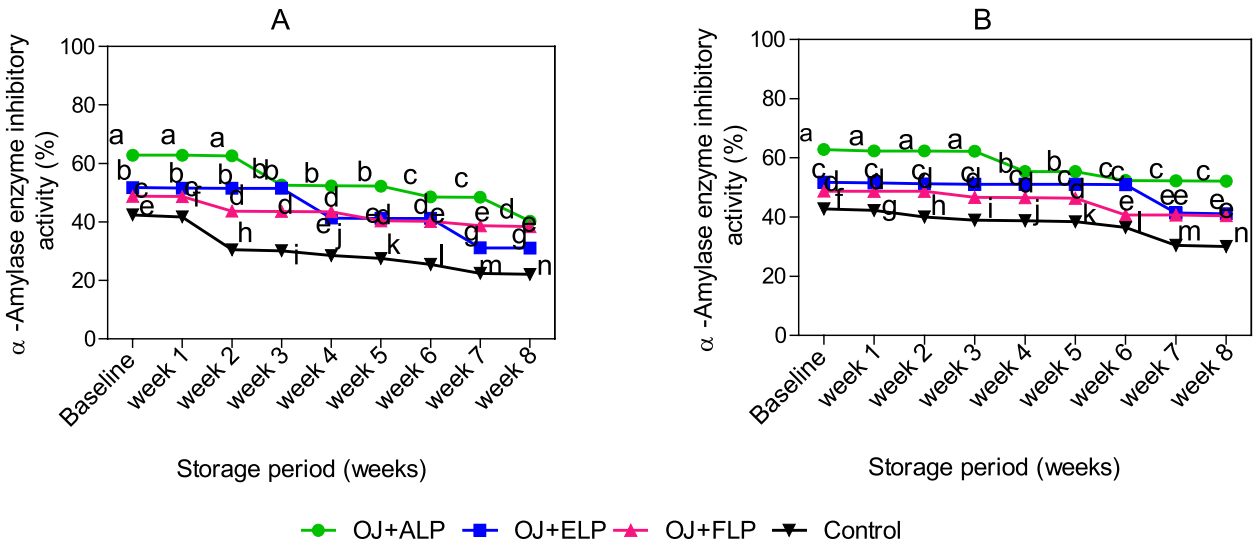


Fig. 4 Graph showing the influence of storage on α-amylase inhibitory activities (%) of samples at Ambient (**A**) and refrigerated storage (**B**) conditions. OJ+ALP: Orange juice incorporated with peptide obtained from Amaranth leafy vegetable; OJ+ELP: Orange juice incorporated with peptide obtained from eggplant leafy vegetable; OJ+FLP: Orange juice incorporated with peptide obtained from fluted pumpkin leafy vegetable; Control: Orange juice only; Amb.: Ambient condition; Ref.: Refrigerated condition

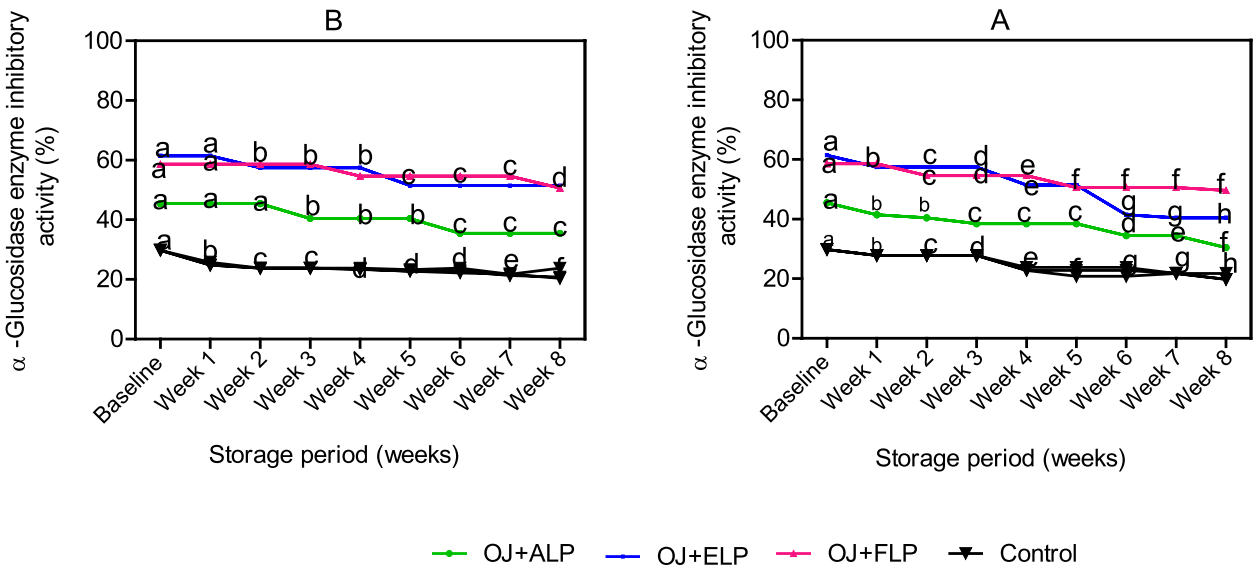


Fig. 5 Graph showing the influence of storage on α-glucosidase inhibitory activities (%) of samples at Ambient (**A**) and refrigerated storage (**B**) conditions

(78%) fruits (Abirami et al. 2014). The differences may be due to the source of the fruit and the degree of ripeness. At ambient storage (Fig. 4A), there were no significant ($p > 0.05$) reductions in the alpha amylase inhibitory activities of the samples until after the 2nd (juice + ALP; juice + ELP;juice + FLP, juice) and 3rd (juice + ELP), week of storage. After a while, the stability of the alpha amylase enzyme was observed in juice + ALP (4th -5th and 6th -7th), juice + ELP (5^{th-}6th and 7th -8th), juice + FLP (3rd -8th) in the ambient condition. The results suggest that minimal decline in the inhibitory activity of amylase enzyme at low temperature (refrigerated storage). Furthermore, at the end of the storage periods, juice samples fortified with peptide exhibited greater amylase enzyme inhibitory activity when compared with the control juice. In this case, it is possible that the peptides added to the juice attached itself to the active site of the enzyme, thereby sustaining the high inhibitory activities of the fortified juice samples as the storage period increased. However, juice sample fortified with ALP showed stronger inhibitory activity when compared with other fortified juice samples, and the values significantly ($p < 0.05$) different from other juice at each storage week and this may be attributable to the history of amylase enzyme inhibitory of the ALP.

In a similar manner, the fortified juice showed greater alpha glucosidase inhibitory activities (45.43–61.45%), when compared with control juice (29.78%) as shown in Fig. 5. The value obtained for the control juice was lower, when compared with the values reported for juice obtained from *Sandoricum koetjape* (46.15%), *Garcinia atroviridis* (65.94%) and *Spondias pinnata* (64.85%) fruits (Sulaimon and Ooi, 2014). Earlier work by Abirami et al. (2014) reported 70, 75 and 73% alpha glucosidase inhibitory activities for juices obtained from *hystrix*, *maxima* red and *maxima* white fruits respectively. The inhibitory activities of alpha glucosidase decreased gradually in both the ambient and refrigerated (Fig. 5) storage conditions, with greater residual inhibitory activities of the enzyme observed in the fortified juice at the end of the 8th week storage periods. Comparing the inhibitory activities of the two enzymes, the results showed that the control juice and the fortified juice exhibited greater alpha amylase inhibitory activities (42.45% &48.74–62.33%) when compared with alpha glucosidase inhibition (29.78% & 45.45–61.45%). The result also showed that juice fortified with ALP had significantly higher alpha glucosidase inhibitory activities than the other fortified juice samples. Previous study by Famuwagun et al. (2020) on the amino acid sequences of peptides identified in the enzymatic hydrolysates of the protein showed that the peptides contained sequences with the ability to inhibit the activities of several enzymes such as angiotensin converting enzymes (ACE) and those related to diabetics (alpha amylase and alpha glucosidase enzymes). By implication, the pattern of results suggests that less of glucose would be liberated from starch during hydrolysis and which may eventually result to less glucose absorption, and this a good management strategy for reducing high blood glucose levels.

Conclusion

The study evaluated the influence of leaf peptides addition on the quality characteristics of orange juice. It also examined the effects of storage temperatures on the stability of the peptides in the resulting fortified juice. The presence of peptides in the juice samples improved the antioxidant properties and enhanced the inhibition of alpha amylase and glucosidase enzymes. During storage, there were changes in the physicochemical properties of the juice samples. The changes were more pronounced in the ambient than refrigerated storage temperatures. The ascorbic acid of the juice samples was observed to decline as the storage period increased. Reduced amounts of oxygen at low temperature storage than at ambient temperature were adduced for the reduction in the loss of ascorbic acid of the refrigerated stored juice samples. The stronger antioxidant properties and greater ascorbic acid retention of the fortified juice compared to the control juice as the storage periods increased was attributed to inhibition of the oxidation of ascorbic acid to dehydroascorbic acid, which may eventually enhance the strong alpha amylase and glucosidase enzymes inhibitory activities of the peptide fortified juice. However, *in-vivo* study of the fortified juice may be necessary to validate the observed claims.

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Authors' contributions
Conceptualization, Akinsola Albert Famuwagun (AAF), Kehinde Adekumbi Taiwo (KAT), Saka Olasunkanmi Gbadamosi (SOG), and Rotimi Emmanuel Aluko (REA); methodology, Akinsola Albert Famuwagun (AAF), Kehinde Adekumbi Taiwo (KAT), Saka Olasunkanmi Gbadamosi (SOG), and Rotimi Emmanuel Aluko (REA); formal analysis, Akinsola Albert Famuwagun (AAF), writing—original draft presentation, Akinsola Albert Famuwagun (AAF); writing—review and editing, Akinsola Albert Famuwagun (AAF), Kehinde Adekumbi Taiwo (KAT), Saka Olasunkanmi Gbadamosi (SOG), Durodoluwa Joseph Oyedele (DJO), Odunayo Clement Adebooye (OCA) and Rotimi Emmanuel Aluko (REA); funding acquisition, Kehinde Adekumbi Taiwo (KAT), Durodoluwa Joseph Oyedele (DJO), Odunayo Clement Adebooye (OCA) and Rotimi Emmanuel Aluko (REA). All authors have read and agreed to the published version ofthe manuscript.

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Availability of data and materials
Data are available on reasonable request.

Declarations

Ethics approval and consent to participate
This study did not involve the use of animal and therefore no ethical form was obtained.

Consent for publication
Not applicable.

Competing interests
Dr. Rotimi Emmanuel Aluko is a member of Editorial Board of *Food Production, Processing and Nutrition* and he was not involved in the journal's review of, or decisions related to this manuscript.

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References

Abirami, A., Nagarani, G., & Siddhuraju, P. (2014). Invitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *citrus hystrix* and *C. maxima* fruits. *Food Science and Human Wellness,3*, 16–25.

Aluko, R. E. (2015). Antihypertensive peptides from food proteins. *Annual Review in Food Science and Technology,6*, 235–262.

Amanda, M., Navam, G., Srinivas, H., Ruiqi, R., Ronny, L., & Han-Seok, S. (2016). Bioactivity of a rice bran–derived peptide and its sensory evaluation and storage stability in orange juice. *Journal of Food Science*. https://doi.org/10.1111/1750-3841.13245

Anushree, B. D., Baul, D., Cyaria G. and Chandra G. (2023). Differential effects of organic inputs on soil fertility and growth of tea plants. *Current Agriculture Research Journal*, 11(2), 520–533. https://doi.org/10.12944/CARJ.11.2.13

Burdulu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering,74*(2), 211–216.

Famuwagun, A. A., Alashi, A. M., Gbadamosi, S. O., Taiwo, K. A., Oyedele, D., Adebooye, O. C., & Aluko, R. E. (2020). Comparative study of the structural and functional properties of protein isolates prepared from edible vegetable leaves. *International Journal of Food Properties,23*(1), 955–970. https://doi.org/10.1080/10942912.2020.1772285

Famuwagun, A. A., Alashi, A. M., Gbadamosi, S. O., Taiwo, K. A., Oyedele, D., Adebooye, O. C., & Aluko, R. E. (2020). Antioxidant and enzymes inhibitory properties of amaranth leaf protein hydrolyzates and ultrafiltration peptide fractions. *Journal of Food Biochemistry*. https://doi.org/10.1111/jfbc.13396

Famuwagun, A. A., Alashi, A. M., Gbadamosi, S. O., Taiwo, K. A., Oyedele, D., Adebooye, O. C., & Aluko, R. E. (2020). In Vitro Characterization of fluted pumpkin leaf protein hydrolysates and ultrafiltration of peptide fractions: antioxidant and enzyme-inhibitory properties. *Polish Journal of Food and Nutritional Sciences,70*(4), 429–443. https://doi.org/10.31883/pjfns/130401

Famuwagun, A. A., Alashi, A. M., Gbadamosi, S. O., Taiwo, K. A., Oyedele, D., Adebooye, O. C., & Aluko, R. E. (2021). Effect of protease type and peptide size on the in vitro antioxidant, antihypertensives and anti-diabetic activities of eggplant leaf protein hydrolysates. *Foods,10*, 1112. https://doi.org/10.3390/foods10051112

Gulcin, I., Mshvildadze, V., Gepdiremen, A., & Elias, R. (2006). The antioxidant activity of a triterpenoid glycoside isolated from the berries ofHedera Colchica: 3-O-(β-d-glucopyranosyl)-hederagenin. *Phytotherapy Research,20*(2), 130–134. https://doi.org/10.1002/ptr.1821

Hollis, J. H., Houchins, J. A., Blumberg, J. B., & Mattes, R. D. (2009). Effects of concord grape juice on appetite, diet, body weight, lipid profile, and antioxidant status of adults. *Journal American College of Nutrition,28*(5), 574–582.

Horáčková, K., Rokytová, K., Bialasová, I., & Sluková, M. (2018). Fruit juices with probiotics – new type of functional foods. Food microbiology and safety. *Czech Journal of Food Science,36*(4), 284–288. https://doi.org/10.17221/39/2018-CJFS

Kim, Y. M., Wang, M. H., & Rhee, H. I. (2004). A novel alpha glucosidaseinhibitor from pine bark. *Carbohydrate Research,339*, 715–727.

Kwon, Y., Apostolidis, E., & Shetty, K. (2006). Inhibitory potential of wine and tea against alpha amylase and alpha glucosidase for management of hypertensive linked to type 2 diabetes. *Journal of Food Biochemistry,32*, 15–31.
Khaw, W., Hasssan, H. & Chan, B. K. (2016). Comparison of total phenolic contents (TPC) and Antioxidant Activities of Fresh Fruit Juices, Commercial 100% Fruit Juices and Fruit Drinks 100%. *Sains Malaysiana,* 45(9), 1319–1327
Lanny, S., & Lie, H. (2014). Study on the kinetics of vitamin c degradation in freshstrawberry. *Procedia Chemistry,9*, 62–68.
Maia, G. A., Monteiro, J. C. S., & Guimaraes, A. C. I. (2001). Estudo Da estabilidade físico-química do suco de caju com alto teor de polpa. *Cienc Technology Alimentarium,21*, 43–46.
Malomo, S. A., He, R., & Aluko, R. E. (2014). Structural and functional properties of hemp seed protein products. *Journal of Food Science,79*, C1512–C1521. https://doi.org/10.1111/1750-3841.12537
Malsha, H., Samarasiri, T. A., Chandrasiri, D. B., Wijesinghe, T. T., & Sanja, P. G. (2019). Antioxidant capacity and total phenolic content variations against *morinda citrifolia* L. fruit juice production methods. *International Journal of Food Engineering,5*, 4.
Marwa, A. R. (2018). Consumer acceptance of orange juice mixed with water mint leave extract. *Recent Patents on Food Nutrition and Agriculture,9*, 50–54.
Mgaya-Kilima, B., Remberg, S. F., Chove, B. E., & Wicklund, T. (2014). Influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends in plastic bottle. *Food Science and Nutrition,1*, 2–10.
Osungbade, O. R., Ikujenlola, A. V., & Gbadamosi, S. O. (2021). Influence of Kersting's Kerstingiella geocarpa) groundnut proteins on the physicochemical, bioactive properties and storage stability of orange juice. *Heliyon,7*, e06246.
Otles, S., & Cagindi, O. (2012). Safety considerations of nutraceuticals and functional foods. In A. McElhatton & P. J. A. Sobral (Eds.), *Novel technologies in food science* (pp. 121–136). Springer.
Pushkala, R., & Srividya, N. (2014). Quality characteristics, nutraceutical profile, and storage stability of aloe gel-papaya functional beverage blend. *International Journal of Food Science,2014*, 847013. https://doi.org/10.1155/2014/847013
Rekha, C., Poornim, G., Manasa, M., Abhipsa, V., Devi, J., Kumar, H., & Kekuda, T. (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science and Transport,1*(2), 303–310.
Renuka, B., Kulkarni, S. G., Vijayanand, P., & Prapulla, S. G. (2009). Fructo-oligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics. *LWT -Food Science and Technology,42*(5), 1031–1033.
Singh, S. K., & Sharma, M. (2017). Review on biochemical changes associated with storage of fruit juice. *Journal of Agricultural Science,9*, 13.
Sridevi, D., Ishwarlal, J., & Sonia, V. L. (2018). Plant sterol-fortified orange juice effectively lowers cholesterol levels in mildly hypercholesterolemic healthy individuals. *Atherosclerosis and Lipoproteins,9*, 5–11. https://doi.org/10.1161/01.ATV.0000120784.08823.99
Sulaimon, F. F. & Ooi, K. L. (2014). Antioxidant and α-glucosidase inhibitory activities of 40 tropical juices from Malaysia and identification of phenolics from the bioactive fruit juices of Barringtonia racemosa and phyllanthus acidus. *Journal of Agricultural and Food Chemistry*, 62(39). https://doi.org/10.1021/jf502912t
Wunwisa, K., & Kamolnate, K. (2010). Sensory characteristics and consumer acceptance of fruit juice containing probioitcs beads in Thailand. *Austin Journal of Technology,14*(1), 33–38.
Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry,111*, 370–376.
Xu, Q., Chen, L. L., Ruan, X., Chen, D., Zhu, A., Chen, C., & Bertrand, D. (2008). The draft genome of sweet orange (*Citrus sinensis*). *Nature Genetics,45*(1), 59–68.
Yuan, M., Xiaocui, Y., Xiufang, B., Fan, S., Zhilin, L., Ming, L., Dongxu, F., Yage, X., & Zhenming, C. (2019). Physicochemical properties and bioactive compounds of fermented pomegranate juice as affected by high-pressure processing and thermal treatment. *International Journal of Food Properties,22*(1), 1250–1269. https://doi.org/10.1080/10942912.2019.1640737
Zhang, J., Han, H., Xia, J., & Gao, M. (2016). Degradation kinetics of vitamin C in orange and orange juice during storage. *Advance Journal of Food Science and Technology,12*(10), 555–561. https://doi.org/10.19026/ajfst.12.3303
Zheng, W.Z. & Wang, S.Y. (2001) Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, 49(11), 5165–5170

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