Heliyon

Received:
13 March 2018
Revised:
24 April 2018
Accepted:
11 June 2018

Cite as: Dinah Kirigia, Traud Winkelmann, Remmy Kasili, Heiko Mibus. Development stage, storage temperature and storage duration influence phytonutrient content in cowpea (*Vigna unguiculata* L. Walp.). Heliyon 4 (2018) e00656. doi: 10.1016/j.heliyon.2018.e00656

Development stage, storage temperature and storage duration influence phytonutrient content in cowpea (*Vigna unguiculata* L. Walp.)

Dinah Kirigia [a,b,*], **Traud Winkelmann** [a], **Remmy Kasili** [c], **Heiko Mibus** [b]

[a] *Leibniz Universität, Hannover, Institute of Horticultural Production Systems, Germany*

[b] *Geisenheim University, Institute of Urban Horticulture and Ornamental Plant Research, Germany*

[c] *Jomo Kenyatta University of Agriculture and Technology, Institute of Biotechnology Research, Kenya*

[*] Corresponding author.

E-mail address: dinahkarimi@gmail.com (D. Kirigia).

Abstract

Cowpea (*Vigna unguiculata*) plays an important role in sustainable food security and livelihood improvement in Sub-Saharan Africa (SSA). The crop is rich in phytonutrients and minerals, which are key in solving malnutrition and hunger crisis, a major challenge in SSA. However, physiological status, storage temperature and duration affect phytonutrient levels and postharvest life of the leafy vegetable. Despite the significant importance of cowpeas, the maturity and postharvest storage effects on quality of the leafy vegetable remains unrevealed. The aim of this study was to analyze the dynamics of phytonutrients in cowpea leaves during development under field conditions in Kenya and in storage. The total carbohydrates (glucose, fructose, sucrose and starch) were highest at 90 d after planting (105.9 $\pm$ 2.5 g kg^{-1}) compared to 30, 60 and 120 d. The total Phenolics (Gallic acid equivalents) increased gradually with age up to 12.0 $\pm$ 0.2 g kg^{-1} by 120 d. Catechin equivalent flavonoids, trolox equivalent antioxidants (TEA) and chlorophyll were highest in concentrations at 60 d after

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

planting with 8.0 ± 0.5 g kg^{-1}, 26.19 ± 0.5 g kg^{-1} and 5.7 ± 0.4 g kg^{-1}, respectively. Quercetin equivalent flavonoids and total carotenoids did not show significant changes with age, while mineral concentration dynamics were specific for each element. Storage of cowpea leaves at room temperature (50−55 % relative humidity) led to a stronger decline of phytonutrients after 4 d, but mostly they remained stable at cold storage (5 °C). Results of this study highlight the importance of developmental stage at harvest, storage conditions and duration for the optimal availability of phytonutrients in freshly consumed leaves and for postharvest management strategies.

Keywords: Nutrition, Food analysis, Food science

1. Introduction

African leafy vegetables (ALVs) hold high potential in solving food insecurity menace and improving livelihoods in Sub-Saharan Africa (SSA) (Grivetti and Ogle, 2017). In addition to lack of enough food (calories), the hidden-hunger crisis due to the deficiency in proteins, minerals and microelements has been a health concern for many centuries in SSA (Okonya and Maass, 2014). This clearly shows the need for a sustainable supply of both quality and quantity of food. Currently, leguminous crops have been identified as the affordable and sustainable source of essential nutrients and low cost proteins for a balanced human diet (Avanza et al., 2013; Goncalves et al., 2016). Cowpea has several agronomic, economic and environmental advantages hence it remains most suitable and highly cultivated legume crop across Africa, Asia and South America (Linguya et al., 2015; Goncalves et al., 2016). The multipurpose crop is suitable for intensification of a sustainable agri-food system in SSA, with the whole above-ground part, being utilized for its leaves, green pods, green beans, mature beans, or processed into paste or flour. The remaining waste of the crop is used as forage, hay or silage for livestock feeding (Goncalves et al., 2016).

Cowpea leaves can be used as fresh green vegetables, fermented or sun-dried (Saidi et al., 2010; Ibrahim et al., 2002; Wafula et al., 2016). The leaves are rich in vitamins, macro and micro minerals, flavonoids, antioxidants, ß-carotene, fatty acids, amino acids, carbohydrates and dietary fibre (Okonya and Maass, 2014; Goncalves et al., 2016). The rich source of phytonutrients in cowpeas makes it one of the most suitable African indigenous crop in the provision of a health balanced diet and solving malnutrition crisis among the resource-constrained households in SSA (Okonya and Maass, 2014). Daily or frequent inclusion of cowpeas in the diets would therefore, not only solve problems with hunger but help in reducing the risk of chronic health conditions. Currently, secondary metabolites such as flavonoids, antioxidants, phenolics and carotenoids in African vegetables have received a lot of research attention for their wide range of pharmacological and

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

biochemical benefits (Moyo et al., 2013; Neugart et al., 2017). They have antioxidative capacity, hence scavenges free radical ions and protect humans against cancer illnesses, and as well the metabolites have anti-inflammatory and antimicrobial properties (Nijveldt et al., 2001). The secondary metabolites also play key roles in the plant defense and protective mechanisms against biotic and abiotic stress (Nijveldt et al., 2001; Akula and Ravishankar, 2011).

Phytonutrient contents in vegetables and fruits are influenced by crop age or developmental stage, storage conditions and duration among other factors. Harvesting stages have been shown to influence postharvest behavior, nutritional quality and shelf life in some vegetables such as leaf amaranthus, spinach and lettuce (Lee and Kader, 2000; Modi, 2007; Oloyede et al., 2013; Spinardi et al., 2016). However, there is little information about effects of age on the nutrient content of many indigenous vegetables. Establishment of maturity stages or indices for harvesting would ensure optimum nutrient accumulation in cowpeas for uptake in human diets and/or postharvest processing. Additionally, storage temperature after harvesting of vegetables directly influences their metabolism. Unfavorable storage conditions and prolonged storage duration have been reported to lead to degradation of chlorophyll, carotenoids, ascorbic acid, antioxidants and carbohydrates on vegetables (Acedo, 2010; Spinardi et al., 2016). The changes of phytonutrients in cowpea leaves during development and after harvesting has not been studied so far. Despite the contribution of cowpeas and other traditional vegetables in household food security and poverty eradication, sufficient research has to date not been conducted to explore their full potential. This study aimed at evaluating the effects of plant age, storage conditions and storage duration on phytonutrient content for quality assurance and postharvest losses management of cowpea leaves.

2. Materials and methods

2.1. Experimental set-up

Seeds of the commonly grown Tanzanian variety *V. unguiculata* cv. Tumaini were kindly provided by World Vegetable Center, Arusha, Tanzania. Field experiments were conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kenya (S01°05.9′ E037 °00.8 and 1476 m above the sea level). Three field experiments were set with randomized complete block design (RCBD) in the period from February to May 2015, November 2015 to February 2016 and August to November 2016. Sowing of cowpeas seeds was done at a spacing of 20 × 30 cm between plants and rows, in 6 plots measuring 10 × 15 m wide. Di-ammonium phosphate ($(NH_4)_2HPO_4$) fertilizer was applied at a rate of 2 g per plant (18 g/m^2) and incorporated into the soil by mixing before placing the seed. Drip-irrigation was done to keep the soil moist. Harvesting was done by picking 2−3 leaves at random from the

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

middle part of the plants at 30, 60, 90 and 120 d after planting (Fig. 1). Plant from outer rows in each plot were not harvested or used in the experiment.

Harvesting was done after 4 h of sunlight, in 6 biological replicates for each experiment, and each replicate consisted of leaves picked from 3 plants at random and pooled together. All the harvested samples were packed in perforated zip-rock polythene bags before storage. The control samples were immediately frozen at −20 °C after harvesting and represented day zero for the storage duration experiments. The other samples were stored in darkness at 5 °C with 80−85 % relative humidity (in refrigerator) and at 20−22 °C (room temperature (RT)) with 50−55 % relative humidity, for 2, 4, 6 and 8 d. The samples were then frozen at −20 °C shortly before freeze-drying. The freeze-dried samples were ground into fine powder and weighed (20−30 mg) into 2 ml Eppendorf tubes for phytonutrient analysis. The data is presented as g kg^{-1} or mg kg^{-1} dry weight (DW) of the leaf material. The main presented and described data in this study are from the field experiments August to November 2016, while few data comprising of mainly carbohydrates, chlorophyll and carotenoids from field experiments February to May 2015 and November 2015 to February 2016, are presented as additional data.

2.2. Carbohydrates analysis

2.2.1. Glucose, fructose and sucrose (GFS)

Glucose, fructose, sucrose (GFS) and starch were analyzed using enzymatic and photometric methods with slight modification as described by Gomez et al. (2007)

Fig. 1. Images of cowpea plants at different days after planting; (a) 30 d, (b) 60 d, (c) 90 d and (d) 120 d.

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

and Zhao et al. (2010). The freeze-dried samples were ground into powder and were weighed into 2 ml Eppendorf tubes (20−30 mg) and used for carbohydrates analysis. Extraction was done with 80 % (v/v) ethanol, incubated in a water bath at 80 °C for 15 min. The ethanol extracts were used for GFS enzymatic assays while the pellet was further processed for starch extraction. For GFS, 20 μl aliquots of the extracts were pipetted into 96 well microplates and placed in an oven at 50 °C for 30 min to evaporate the ethanol. The dried wells were then eluted with an equal volume of double distilled water (ddH_20). The ddH_2O was used as blank and the standards for the calibration curve were made from serial dilutions of 1.0 g L^{-1} glucose. A 96 well microplate was loaded with samples in triplicates, and analysis of GFS was done sequentially starting with glucose, followed by fructose and finally sucrose.

A buffer (100 ml) containing 14 g of triethonalamine hydrochloride (Ref 4804, Roth) and 250 mg of $MgSO_4 \times 7H_2O$ (pH 7.6) (TRA buffer) was prepared. The TRA buffer (10 ml) was then used to dissolve a mixture of 100 g $NaHCO_3$, 100 g ATP (Ref 10519987001, Roche) and 20 g NADP (Ref AE13.2, Roth). The mixture of $NaHCO_3$, ATP and NADP in TRA buffer was then pipetted (100 μl each) into the wells. Ten microliter of hexokinase/glucose 6-phosphate dehydrogenase (HK/G6P-DH) (Roche, 10737275001), was then added to the wells and mixed by shaking gently before incubating for 30 min at 30 °C. Glucose absorbance was read at 340 nm on the Versamax® MP photometric reader (Molecular devices, Canada). The phosphoglucose isomerase (PGI) (Ref 10128139001, Roche) (0.25 U) was then added to the wells (10 μl), mixed by shaking gently, incubated for 30 min at 30 °C and absorbance reading for fructose was taken at 340 nm. For sucrose analysis, Invertase from baker's yeast (960 U, Lot 21K7435, Sigma-Aldrich) was dissolved in citric buffer (citric acid and Tri-sodium citrate, pH 4.6) and 20 μl was added to the wells and mixed gently, then incubated at 30 °C for 45−60 min. The final absorbance reading for sucrose was taken at 340 nm. Calculations of each of the GFS was done as described by Gomez et al. (2007).

2.2.2. Starch

The pellet derived after GFS extraction (Section 2.2.1) was washed with ddH_2O, dried in an oven at 60 °C and used for starch analysis with slight modifications as described by Gomez et al. (2007) and Zhao et al. (2010). Starch was hydrolyzed by adding 500 μl of 0.5 M NaOH and incubated at 60 °C on a shaker for 1 h. The pellet was then cooled and neutralized with 25 μl 1 M acetic acid and 475 μl ddH2O and mixed by vortexing followed by spinning at 4200 × g for 10 min. From the supernatant, 10 μl was transferred to a clear 96-well microplate in triplicates. The Amyloglucosidae (AG) (9 U) was weighed (4 mg) and dissolved in 1.4 ml citric buffer (Section 2.2.1), from which 20 μl was added to the wells, and

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

incubated for 30 min at 60 °C. The plate was cooled and 100 μl of TRA buffer (Section 2.2.1) added followed by hexokinase/glucose 6-phosphate dehydrogenase (HK/G6P-DH) (Section 2.2.1) (10 μl), and incubated for 30 min at 30 °C. The absorbance was then recorded at 340 nm and the calculations were based on the calibration curve equation derived from glucose standards as described by Gomez et al. (2007).

2.3. Total phenolics content

Determination of total phenolics content using the Folin-Ciocalteu (F-C) assay was done as described by Ainsworth and Gillespie (2007). Finely ground freeze-dried samples (20−30 mg) were weighed into 2 ml tubes. The total phenolic compounds were extracted with 2 ml 97 % (v/v) methanol for two days at room temperature (RT) in darkness. Ten percent (v/v) of Folin & Ciocalteu's phenol reagent (F-C) (Ref F9252, Sigma- Aldrich) was added to 100 μl of the plant extract, and mixed thoroughly by vortexing. This was followed by the addition of 800 μl 700 mM Na_2CO_3 and incubation for 2 h at RT. Samples were spinned at 15800 × g for 1 min and 200 μl of supernatant was transferred to a clear 96-well microplate in triplicates. The absorbance of each well was measured and recorded at 765 nm using a Tecan microplate reader (Infinite® 200). Gallic acid was used to prepare the calibration curve. The calculated amount of total phenolic content was expressed as gallic acid equivalents per gram of dry weight.

2.4. Flavonoids

The aluminium complexation reaction assay was carried out for catechin and quercetin equivalents as described by Eghdami and Sadeghi (2010). For catechin, 100 μl of the methanol extracts described in Section 2.3 were mixed with 300 μl distilled water and 30 μl 5 % (w/v) $NaNO_2$, and incubated for 5 min. Thirty microliters of 10 % (w/v) aluminum chloride ($AlCl_3$) were added followed by another incubation for 5 min. The reaction mixture was then treated with 200 μl 1M NaOH and 340 μl distilled H_2O to reach a final volume of 1000 μl. Aliquots of 200 μl from the supernatant of each sample were pipetted to a clear 96-well microplate in triplicates. Absorbance at 510 nm was measured with a Tecan microplate reader (Infinite® 200). The calibration curve was done with catechin serial dilutions of 12.5, 25, 50, 100, 250 g L^{-1}. The calculated amount of catechin and its derivatives were then expressed as catechin equivalents in g kg^{-1}. In the quercetin analysis, 100 μl of the extract was mixed with 300 μl of 97 % methanol, plus 20 μl of 10 % $AlCl_3$ and 20 μl of 1M NaOAc. Finally, 560 μl H_2O were added to bring the total volume to 1000 μl. Aliquots of 200 μl from the supernatant from each sample were pipetted to a clear 96-well microplate in triplicates. Absorbance at 415 nm was measured with a Tecan microplate reader (Infinite® 200). The calculated amount of quercetin flavonoids was then expressed as quercetin equivalents.

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2.5. Antioxidants

Antioxidants were analyzed using the Trolox equivalent antioxidants capacity (TEAC) assay against 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS• + radical cation) (Ref A1888-1G, Sigma-Aldrich) as described by Miller et al. (1993), and with slight modifications as suggested by Tyrakowska et al. (1999). The principle of the method was based on the ability of the antioxidants to scavenge the ABTS•+ radical cation in comparison to the antioxidative capacity of an artificial and water-soluble vitamin E derivative, Trolox® (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (Ref 1238813-1G, Sigma-Aldrich). The pre-formed blue/green radical of ABTS• + cation was generated by addition of potassium persulfate to a solution of the ABTS diammonium salt, which has an absorption maximum at 734 nm. The ABTS• + radical cation is reduced in presence of hydrogen-donating antioxidants leading to decolorization of the blue/green-colored solution. The decolorization of the ABTS• + radical cation is then a measure of the antioxidant capacity, which is given in Trolox equivalents (Miller et al., 1993). In the TEAC assay, the methanol extracts described in Section 2.3 above, were diluted 1:20 with 50 mM of phosphate buffer (41 mM Na_2HPO_4 and 9 mM NaH_2PO_4, pH 7.2). Sample aliquots of 10 μL were pipetted into 96 well microplates with three replicates each. ABTS stock solution (7 mM) was prepared and incubated overnight at RT in darkness where an intensely blue colored complex developed. An ABTS working solution (about 140 μM) was prepared by diluting with phosphate buffer (1:50 to 1:70) until the extinction at 734 nm was between 0.7 and 0.8. The diluted ABTS solution (190 μl) was then added to the samples by the injector in the Tecan microplate reader, and after 5 min the absorbance was recorded at 734 nm. All analyses were carried out in triplicates. The calibration and regression lines were received from the Trolox® stock solution in a dilution series of 0.025−0.4 mM in phosphate buffer.

2.6. Chlorophyll and carotenoids

The total chlorophyll and carotenoids content were determined in cowpea leaves by UV-VIS spectroscopy, as described by Lichtenthaler (1987). The freeze-dried material was ground into powder, 20−30 mg weighed into Eppendorf tubes with 6 replicates, and 4.5 ml of 90 % (v/v) ethanol extractions were done for the pigments. Samples were then vortexed and spinned at 15800 × g for 1 min and 200 μl of the supernatant was aliquoted to the 96 well plate in triplicates and covered with aluminium foil. The absorbance readings were done at 470 nm, 648 nm and 664 nm (as well as 750 nm to check for impurities) using the Tecan microplate reader (infinite® M200). Calculations for total chlorophyll and carotenoids were done according to the formula described by Lichtenthaler (1987).

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2.7. Mineral analysis

Finely ground plant samples were weighed into 100 ml test tubes (250 mg) in three biological replicates, and each with 3 technical replicates. Digestion of plant material was done with lithium sulfate buffer (0.48 g of selenium powder, 14 g of lithium sulfate monohydrate 99.99 Suprapur® (CAS 10102-25-7, Sigma-Aldrich), 420 ml sulfuric acid 95−97 %, and 330 ml hydrogen peroxide 30 % stabilized). This buffer was prepared by dissolving selenium and lithium sulfate in 3 L of sulfuric acid in a PE beaker while placed in a bowl of cold water. Hydrogen peroxide was then added slowly while stirring, and the liquid became clear after cooling. Ten microlitre of the buffer was then added by tilting pipette to the samples and heated in the Turbotherm apparatus Gerhardt (Gerhardt, analytical systems, Königswinter, Germany). After cooling, the samples were filled with deionized water to 50 ml, mixed on a test tube shaker and filtered through a pleated filter (Munktell 3/N) in PE bottles. The measurement of Ca, K, Mg, Fe, Zn, Mn, P and Cu elements was carried out by inductively coupled plasma optical emission spectrometry (ICP-OES), while nitrates was quantified through flow injection analysis (FIA) method (Jones, 2001; Brabcová et al., 2003).

2.8. Statistical analysis

All the acquired data were subjected to normality test before analysis of variance was conducted using the SPSS (version 20) statistical software. The means were compared using Tukey test at $p < 0.05$. The data were presented as means ± standard errors g kg^{-1} or mg kg^{-1} dry weight.

3. Results

The phytonutrient analysis were done from cowpeas samples harvested at 30, 60, 90 and 120 d after planting (Fig. 1). Cowpeas was at vegetative phase from 30 to 60 d, while at 90 d, the crop was at reproductive phase with over 90 % of the plants having flowers or already developing young pods (Fig. 1). By 120 d, plants already had mature pods and most of the older leaves were already turning yellow as a sign of senescence (Fig. 1). In this study, glucose concentration was significantly lower at 30 d (1.5 ± 0.15 g kg^{-1}) than at 60, 90 and 120 d (9.9 ± 0.5, 11.0 ± 2.7, 10.2 ± 1.7 g kg^{-1}), respectively (Fig. 2). Fructose and sucrose concentrations were highest at 90 d (9.5 ± 0.03 and 48.12 ± 5.2 g kg^{-1}), respectively, while the starch concentration did not show any significant difference between the developmental stages. The total of all the four sugars indicated highest carbohydrate contents at 90 d after planting (Fig. 2). From the field experiments done from February to May 2015 and November 2015 to February 2016, although there were changes with specific sugar quantities with age, the total carbohydrates remained highest at 90 d after planting

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

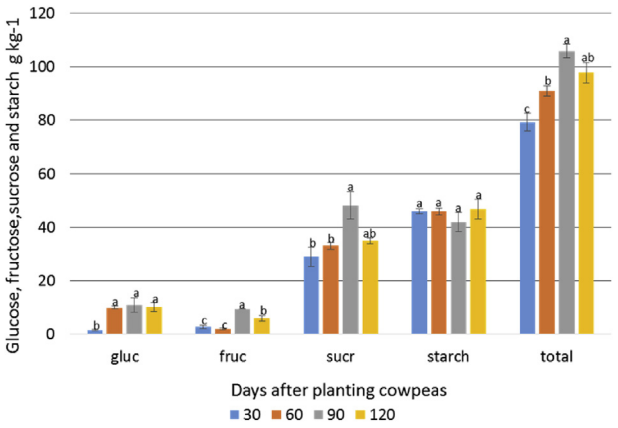

Fig. 2. Glucose (gluc), fructose (fruc), sucrose (sucr) and starch concentrations and their totals in cowpea leaves harvested 30, 60, 90 and 120 d after planting. The data is from field experiment August to November 2016. The values represent means ± standard error. Means labeled with the same letters within each sugar were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

compared to other development stages (Fig. 3). The storage experiments were conducted from 60 d old plants from the field experiments of August to November 2016. During storage at 5 °C and 80−85 % RH, there were no pronounced changes in total sugars and sucrose concentrations, while on the other hand the fructose and glucose increased as the starch content decreased after 6 d of storage (Fig. 4). Storage of cowpea leaves at RT (20−25 °C) at 55−60 % RH led to a significant decrease in concentrations of all three sugars with the steepest reduction being observed after 4 d of storage (Fig. 4). The carbohydrates declined by 81 % if stored under RT as compared to 16 % at cold storage (Fig. 4). The same phenomena of carbohydrates changes was observed during a repeat of storage experiment at 90 d after planting (Fig. 5).

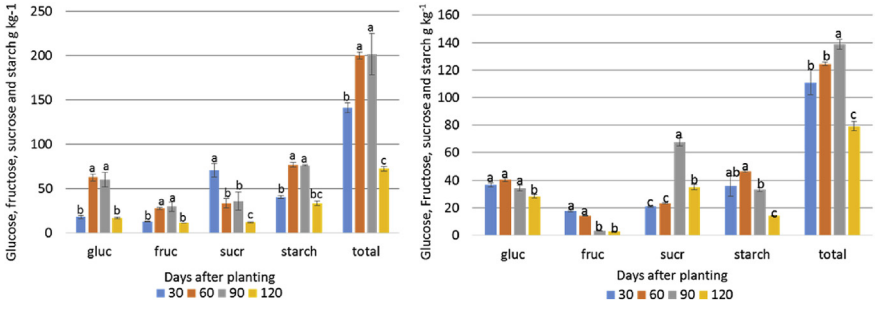

Fig. 3. A = Field experiments February to May 2015 and B = Field experiments November 2015 to February 2016: Glucose (gluc), fructose (fruc), sucrose (sucr) and starch concentrations and their totals in cowpea leaves harvested 30, 60, 90 and 120 d after planting. The values represent means ± standard error. Means labeled with the same letters within each sugar were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

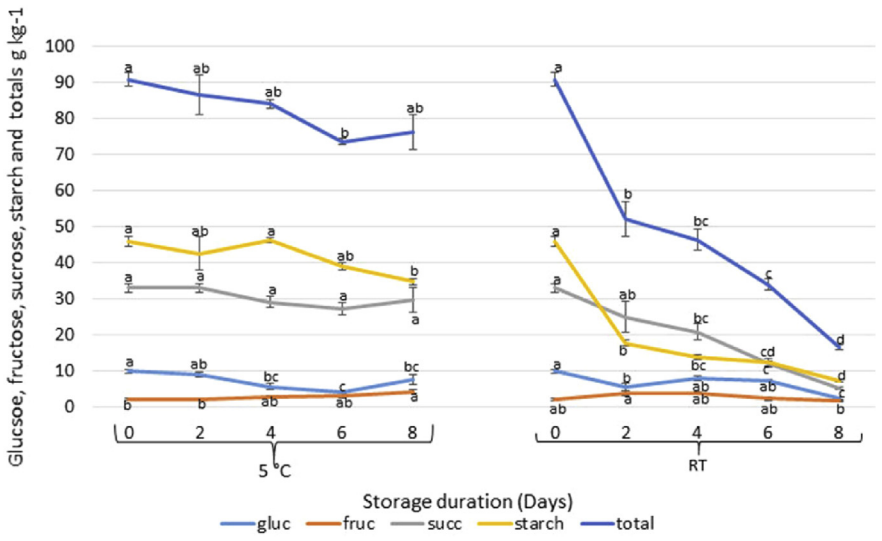

Fig. 4. Glucose (gluc), fructose (fruc), sucrose (sucr) and starch and their totals in cowpea leaves harvested at 60 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within each sugar were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

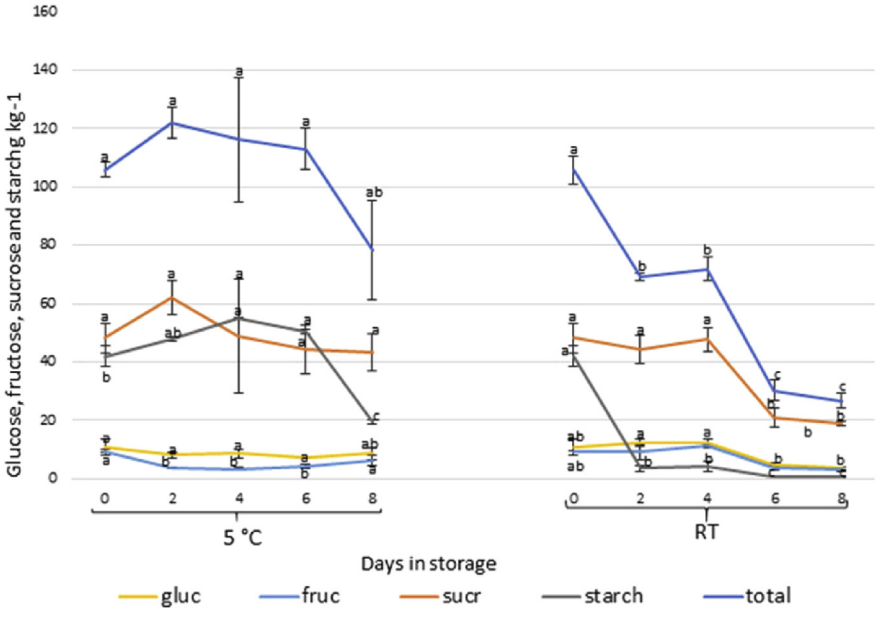

Fig. 5. Glucose (gluc), fructose (fruc), sucrose (sucr) and starch and their totals in cowpea leaves harvested at 90 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within each sugar were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

The dynamics of secondary metabolites during development and storage were also investigated. Quercetin equivalent flavonoids were detected in higher concentrations than catechin equivalent flavonoids in cowpea leaves (Fig. 6). For quercetin, no significant differences in concentration were observed among the different plant ages

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

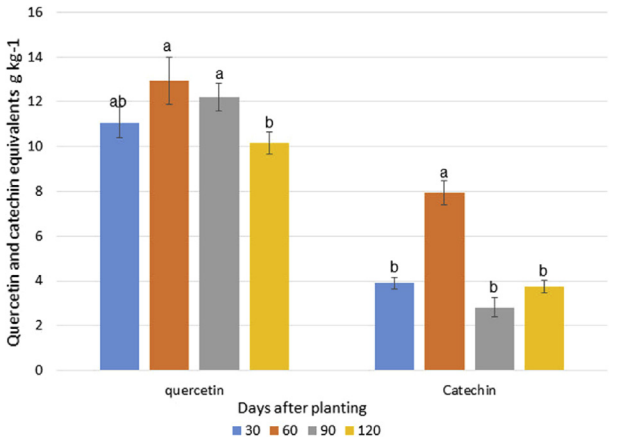

Fig. 6. Flavonoids (quercetin and catechin equivalents) content in cowpea leaves harvested at 30, 60, 90 and 120 d after planting. The values represent means ± standard error. Means labeled with the same letters within a metabolite group were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

(Fig. 6). In contrast, catechin was detected in significantly higher concentrations at 60 d after planting (8.0 g kg^{-1}) compared to 30, 90 and 120 d (3.9 ± 0.3, 2.8 ± 0.4 and 3.7 ± 0.3 g kg^{-1}, respectively) (Fig. 6). In both storage temperatures, there was degradation of flavonoids which was more pronounced for quercetin than for catechin flavonoids (Fig. 7). Between storage conditions, only minor differences were observed during the first 6 d of storage, whereas thereafter, higher storage

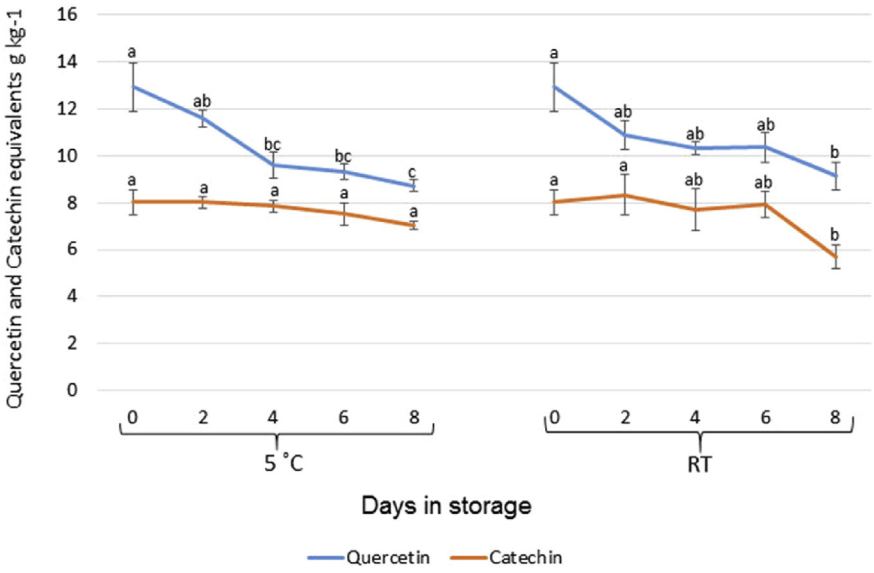

Fig. 7. Flavonoids (quercetin and catechin equivalents) in Cowpeas leaves harvested at 60 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within a metabolite were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

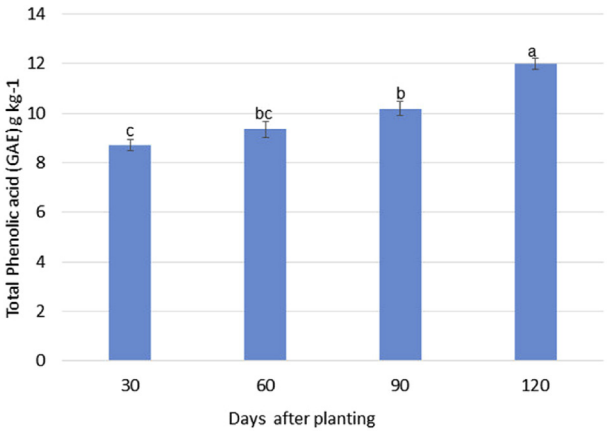

Fig. 8. Total phenolic (Gallic acid equivalents (GAE) content in cowpea leaves harvested 30, 60, 90 and 120 d after planting. The values represent means ± standard error. Means labeled with the same letters were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

temperatures resulted in a steeper decrease in concentration of both groups of flavonoids (Fig. 7).

The total phenolics content gradually increased with plant age in cowpeas, 8.7 ± 0.2, 9.3 ± 0.3, 10.2 ± 0.3 and 12.0 ± 0.2 g kg^{-1} for 30, 60, 90 and 120 d after planting respectively (Fig. 8). During storage, there was 34.5 % decline of the phenolics at RT and 47 % decline (from 10.6 ± 0.6 to 5.6 ± 0.2 g kg^{-1}) was observed after storing at 5 °C for 8 d (Fig. 9). This signifies losses of phenolics in longer storage duration despite the temperature. The antioxidants just like total phenolics increased with plant age and were significantly higher after 60 d (26.2 ± 0.6 g kg^{-1}) than at younger stages, 30 d (22.3 ± 0.5 g kg^{-1}) (Fig. 10). The antioxidants appeared stable up to 6

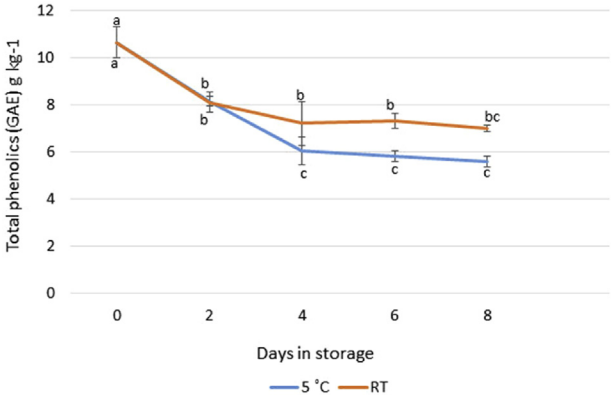

Fig. 9. Total phenolic (GAE) contents in Cowpeas leaves harvested at 60 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within a metabolite were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

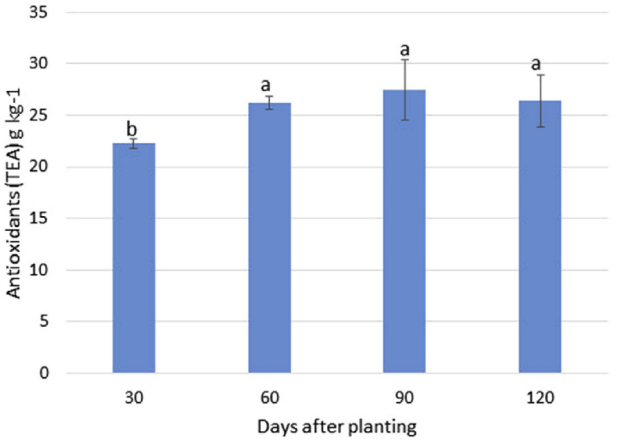

Fig. 10. Trolox equivalents antioxidants (TEA) content in cowpeas leaves harvested 30, 60, 90 and 120 d after planting. The values represent means ± standard error. Means labeled with the same letters were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

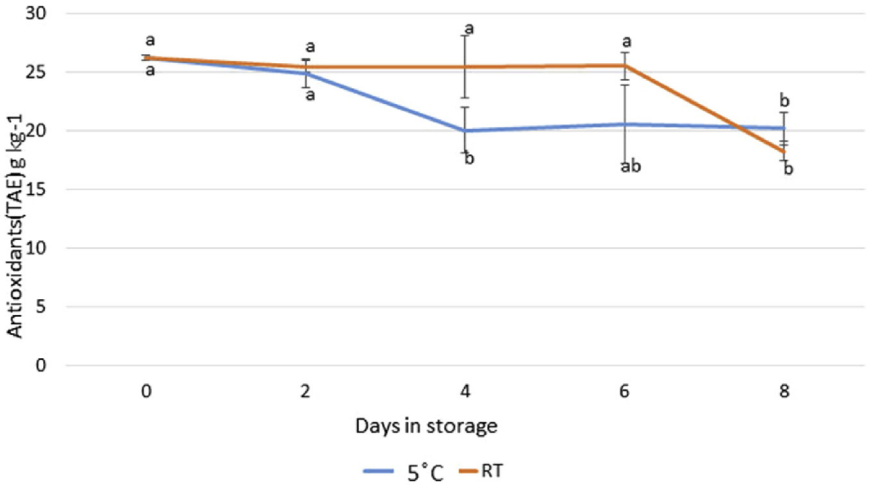

Fig. 11. Antioxidants (TEA) content in cowpeas leaves harvested at 60 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within a metabolite were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

d in storage at RT, but a significant decline was observed by 8 d of storage (Fig. 11). A slight decline was observed during storage at cold temperatures, after 4 d and later remained stable (Fig. 11).

The total chlorophyll content during plant development increased significantly between 30 d (4.3 ± 0.2 g kg^{-1}) and 60 d (5.7 ± 0.4 g kg^{-1}) and remained stable thereafter (Fig. 12; A). Carotenoids did not show any significant difference between the four development stages of cowpeas and the concentration ranged from 0.8 to 0.9 g kg^{-1} (Fig. 12; A). However, the field experiments in February to May 2015 and November 2015 to February 2016, indicated high chlorophyll content 60 d after

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

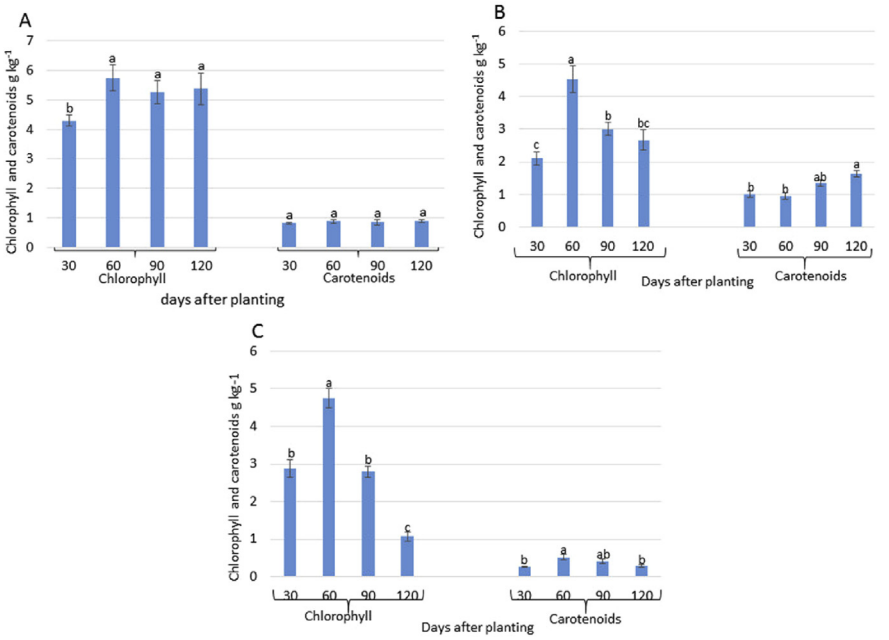

Fig. 12. Chlorophyll and carotenoid concentration in cowpeas leaves harvested 30, 60, 90 and 120 d after planting. A = Field experiments August to November 2016, B = Field experiments February to May 2015, and C = Field experiments November 2015 to February 2016. The values represent means ± standard error. Means labeled with the same letters were not significantly different at $p < 0.05$ using Tukey's test (n = 6).

planting and an increase of carotenoids with age (Fig. 12; B&C). The chlorophyll later decreased in 90−120 d after planting (Fig. 12; B&C). Storage of cowpeas harvested at 60 dap at 5 °C indicated no significant difference in chlorophylls and carotenoids after storing for 0, 2, 4, 6 and 8 d (Fig. 13). On the other hand storage at RT led to a 49 % decline of the total chlorophyll by day eight. The carotenoids quantities were not significantly affected by RT storage at 60 d after planting

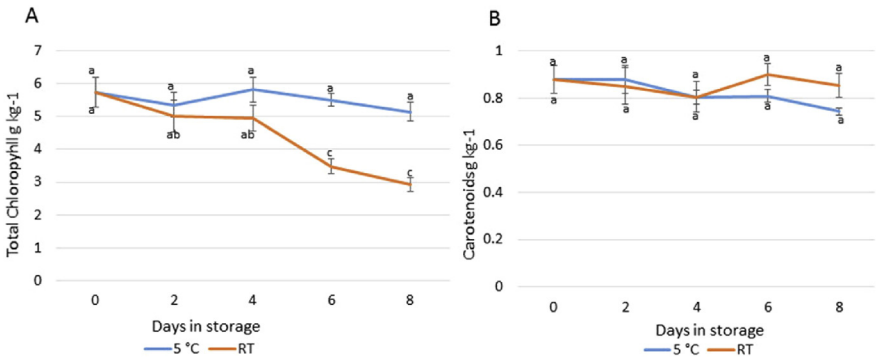

Fig. 13. A = Chlorophyll and B = carotenoid (g kg^{-1}) concentration in cowpea leaves harvested at 60 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within a group and storage condition were not significantly different at $p < 0.05$ using Tukey's test (n = 6).

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(Fig. 13). The same phenomena of chlorophyll and carotenoids changes were observed during a repeat of the storage experiment from cowpea leaves harvested at 30, 90 and 120 d after planting, although slight changes of carotenoids were seen at young leaves at 30 d after planting (Table 1). The greatest percentage losses of chlorophyll was observed at 90 and 120 d after planting (67.9 and 68.5 %), compared to 39.5 % at 30 d after planting (Table 1).

Results in this study indicated that cowpeas is a rich source of both macro and microelements mineral contents. The dynamics of mineral concentrations in cowpea leaves during development was specific for different elements (Table 2). Minerals such as phosphorous, zinc, potassium and magnesium, nitrates and copper were found to be relatively stable at different development stages. Iron and manganese were higher at later development stages (559.9 ± 72 and 327.5 ± 11 mg kg^{-1}), while the calcium concentration was highest at 30 d (19 g kg^{-1}) and indicated a decline thereafter (Table 2). During storage, the minerals did not show significant changes (data is not presented).

Table 1. Chlorophyll and carotenoid (g kg^{-1}) concentration in cowpea leaves harvested at 30, 90 and 120 d after planting and stored at 5 °C and RT for 0, 2, 4, 6 and 8 d. The values represent means ± standard error. Means labeled with the same letters within a group and storage condition were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

Days after planting	Days in storage	Chlorophyll		Carotenoids	
		5 °C	RT	5 °C	RT
30	0	4.3 ± 0.2a	4.3 ± 0.2a	0.8 ± 0.0a	0.8 ± 0.0ab
	2	4.1 ± 0.1a	4.4 ± 0.2a	0.8 ± 0.0a	0.9 ± 0.1a
	4	4.0 ± 0.2a	4.2 ± 0.3a	0.8 ± 0.0a	0.9 ± 0.1a
	6	4.2 ± 0.1a	2.9 ± 0.5ab	0.7 ± 0.0a	0.7 ± 0.0b
	8	4.1 ± 0.2a	2.6 ± 0.8ab	0.8 ± 0.0a	0.7 ± 0.1b
90	0	5.3 ± 0.4a	5.3 ± 0.4a	0.9 ± 0.1a	0.9 ± 0.1a
	2	5.1 ± 0.4a	5.5 ± 0.6a	0.8 ± 0.1a	0.9 ± 0.1a
	4	4.9 ± 0.6a	3.4 ± 0.3b	0.6 ± 0.0a	0.8 ± 0.1ab
	6	4.8 ± 0.3a	1.9 ± 0.3b	0.7 ± 0.0a	0.7 ± 0.0b
	8	4.9 ± 0.2a	1.7 ± 0.2b	0.7 ± 0.0a	0.6 ± 0.0b
120	0	5.4 ± 0.5a	5.4 ± 0.5a	0.9 ± 0.0a	0.9 ± 0.0a
	2	5.0 ± 0.4a	4.6 ± 1.1ab	0.9 ± 0.0a	0.7 ± 0.2b
	4	5.5 ± 0.2a	4.0 ± 0.8ab	0.9 ± 0.1a	0.6 ± 0.1b
	6	5.2 ± 0.3a	2.3 ± 0.5ab	0.8 ± 0.0a	0.7 ± 0.1b
	8	5.3 ± 0.3a	1.7 ± 0.4b	1.1 ± 0.3a	0.7 ± 0.1b

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 2. Mineral content in cowpea leaves harvested at 30, 60, 90 and 120 d after planting. The values represent means ± standard error. Means labeled with the same letters within a column were not significantly different at $p < 0.05$ using Tukey's test ($n = 6$).

dap	N g kg^{-1}	P g kg^{-1}	K g kg^{-1}	Ca g kg^{-1}	Mg g kg^{-1}	Fe mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}	Cu mg kg^{-1}
30	41.2 ± 0.3b	3.1 ± 0.2a	26.0 ± 1.8a	19.0 ± 0.6a	3.9 ± 0.1a	305.4 ± 4.3b	29.3 ± 3.8a	187.4 ± 10.0b	22.7 ± 3.7a
60	44.3 ± 1.3ab	3.5 ± 0.0a	24.5 ± 0.2a	14.2 ± 0.1b	3.3 ± 0.1b	419.5 ± 16ab	32.2 ± 0.1a	275.1 ± 11.9a	14.9 ± 0.6a
90	41.2 ± 2.2b	2.7 ± 0.3a	20.5 ± 0.3b	12.1 ± 0.8b	2.5 ± 0.2b	371.9 ± 28b	24.6 ± 1.3a	218.4 ± 16.3b	14.7 ± 0.3a
120	47.3 ± 0.7a	3.4 ± 0.2a	23.5 ± 1.0ab	14.2 ± 1.1b	3.0 ± 0.0ab	559.9 ± 71.7a	33.2 ± 0.2a	327.5 ± 10.9a	17.2 ± 1.5a

4. Discussion

4.1. Carbohydrates

Carbohydrates are the major products of photosynthesis and play a central role in driving diverse biological processes from embryogenesis to senescence, by supporting and integrating functions and actions of internal and external regulatory signal. In general, the highest total concentration of all carbohydrates analyzed (105.9 ± 2.5 g kg^{-1}) was detected at 90 d after planting in cowpea leaves (Fig. 2). This high concentration of total sugars at 90 d can be attributed to the well-developed canopy of leaves presenting a high light-exposed surface area and hence a high photosynthetic capacity leading to an accumulation of photosynthetic assimilates. By 120 d, a slight decline in sugar concentration was noticed possibly due to translocation of much sugars from the leaves to the pods, which can be considered as sinks for carbohydrates at this stage (Lemoine et al., 2013). Alternatively, there was probably reduced photosynthetic potential due to aging leaves at 120 d. Previous studies have indicated a cross-link between various metabolites and phytohormones and the regulation of sugar metabolism and transport (Eveland and Jackson, 2012), which could also explain the variation of sugar concentration over development period as observed in this experiment. Sucrose being the major sugar transported from source to sink via the phloem was highest in concentration in the leaves at 90 d, which can be attributed to the development of many fully mature leaves, high chlorophyll content and other signal transductions derived by transition of the crop development phases (Lemoine et al., 2013). The levels of glucose, fructose, sucrose and starch indicated variations and fluctuations during the three experiments and this is due to changes in temperatures, light intensity and possibly slight variations in production conditions. However, in all the cases, carbohydrates contents remained higher 90 d after planting.

During storage at 5 °C and 80−85 % RH, there were no pronounced changes in total sugars and sucrose concentrations, while on the other hand the fructose and glucose increased as starch content decreased after 6 d of storage (Figs. 4 and 5). This can be attributed to the deprivation of simple sugars as a result of metabolic activities still going on in the cells, hence a conversion of starch back to glucose. The degradation

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

of starch occurs when there is glucose deficit and helps in maintaining plants metabolism (Smith et al., 2005; Granot et al., 2014). During storage of plant parts at higher temperatures, the respiration rate and most metabolic processes are higher resulting in faster degradation of sugars to provide energy. This explains the sudden decline of starch after two days in storage at RT, which is converted to monosaccharides, presumably causing the stable contents of sugars between 2 and 4 d. The degradation of carbohydrates during storage consequently leads to spoilage of vegetables and loss of good quality attributes.

4.2. Flavonoids, total phenolics and antioxidants

Plant secondary metabolites, such as phenolics, flavonoids and antioxidants play important roles in the human diet and plant development (Wojakowska et al., 2013). Flavonoids and their conjugates have previously been reported to be involved in defense mechanisms against pathogenic attacks in *Fabaceae* species (Akula and Ravishankar, 2011). This study reveals higher concentrations of quercetin equivalent flavonoids in cowpeas leaves than catechin equivalents (Fig. 6). For quercetin, no significant differences in concentration were observed among the different plant ages. The higher levels of catechin flavonoids after 60 d implies that plants synthesize species-specific secondary metabolites after recognition and perception of various signals. These signals can be derived from external biotic and abiotic factors as well as internal factors such as plant development stages (Grotewold, 2005; Veitch, 2007).

The catechin levels are in the same range as those detected in other leafy vegetables such as *A. hybridus, C. olitorius, B. pilosa, C. gynandra, G. parviflora* and *L. sativa* (Chipurura, 2010). Previous studies show that the influence of age on secondary metabolite composition was specific to different crops (Bergqust et al., 2005; Akula and Ravishankar, 2011; Ghasemzadeh et al., 2014). This indicates that different crops respond differently to various factors that either upregulate or downregulate production of secondary metabolites as well as their degradation. Oloyede et al. (2013), reported an increase in antioxidants, flavonoids and phenolic contents at 6 and 5 weeks after planting of *Amaranthus cruentus* compared to 4 and 3 weeks. On the other hand phenolics, flavonoids and antioxidants decreased with age in *Celosia argentea* (Oloyede et al., 2013). However, the three metabolites are lower in cowpeas as indicated in this study compared to those reported in *Amaranthus cruentus* (Oloyede et al., 2013). In both storage temperatures, there was degradation of flavonoids which was more pronounced for quercetin than for catechin flavonoids (Fig. 7). Between the two storage conditions, only minor differences were observed during the first six days of storage, whereas thereafter, higher storage temperatures resulted in a steeper decrease in concentration of both groups of flavonoids. This indicates that the long storage duration, irrespective of temperature, leads to losses of

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

secondary metabolites. The stability of flavonoids has been reported on baby spinach during the normal retail storage conditions, and this correlates with our findings up to six days (Bergqust et al., 2005). The researchers however, indicated that the changes during storage is specific for different types of flavonoids (Bergqust et al., 2005).

The total phenolics content in cowpeas gradually increased with the crop maturity (Fig. 8). Increase in total phenolics with age has been reported in various crops such as *Amaranthus cruentus* (Oloyede et al., 2013). Longer storage duration of cowpeas led to a decline of total phenolics although to a higher extent at cold storage than at RT. A decline in phenolic contents at cold storage and an increase during storage at RT has been reported on other leafy vegetables such as *Amaranthus caudatus, Amaranthus viridis, Moringa oleifera* and in baby lettuce among others (Mathiventhan and Sivakanesan, 2013). Therefore, although total phenolics are affected by various biotic and abiotic factors, the stability during storage is variety or species dependent (Mathiventhan and Sivakanesan, 2013). Since the phenolics play roles in plant defense, the stability during storage at RT up to 8 d could be as a result of continuous phenols build up as a physiological response to infection and damage of the leave cells and oxidative stress after harvesting.

The antioxidants just like other secondary metabolites increased with plant age and were significantly higher after 60 d than at younger stages 30 d (Fig. 10). In general, the antioxidative capacity of cowpeas leaves seemed stable up to 6 d despite the storage temperature, and was only affected by prolonged storage duration at RT after 8 d (Fig. 11). Mathiventhan and Sivakanesan, 2013, pointed out an increase of antioxidants in storage both at RT and at 4 °C and in *Amaranthus caudatus, Amaranthus viridis and Moringa oleifera*. These researchers indicated that the increase of both total phenolics and antioxidants during storage could be due to a continuous breakdown of tannins to free phenolics. Therefore this could also explain the stability of this compounds observed in this study.

4.3. Chlorophylls and carotenoids

The physical characteristics of vegetables after harvesting can easily be determined by greenness and freshness, key factors that affect the market. Therefore chlorophyll quantification can be used as a marker to evaluate the status and quality of green leafy vegetables during storage and development. Results in this study indicated higher chlorophyll contents after 60 d than at younger stages (30 d), while carotenoids did not show any significant difference between the four development stages of cowpeas (Fig. 12). However, in the preliminary experiments and increase in carotenoids with age was observed while chlorophyll remained higher 60 d after planting. The differences in the three experiments could be due to different radiation and temperature. Storage of cowpeas at 5 °C indicated no significant difference in chlorophyll and carotenoids after storing for 0, 2, 4, 6 and 8 d (Fig. 13). This shows that

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

cold storage preserves the chlorophyll and carotenoids integrity in cowpeas hence quality can be enhanced for a longer duration. On the other hand, storage at RT led to a significant decline of chlorophyll, but carotenoid concentration remained stable, and thus resulted in a significantly lower chlorophyll to carotenoid ratio after 8 d in storage. The low chlorophyll to carotenoid ratio indicates degradation of chlorophyll at higher temperatures and longer storage duration. This is possibly due to break down of various physiological processes and increase in ethylene gas production leading to chlorophyll degradation. Previous reports have highlighted various enzymes such as chlorophyll oxidase, peroxidase-hydrogen peroxide and lipid peroxidation to be involved in chlorophyll degradation in leafy vegetables such as spinach (Yamauchi and Watada, 1991; Limantara et al., 2015). The decline of the chlorophyll content can also be associated with the wilting, yellowing and browning which was observed after 6 d of storage at RT. Low temperatures are known to slow down leaf metabolism hence preserves the color and quality and this explains why degradation of chlorophyll was not observed at low temperature storage.

4.4. Minerals

Mineral content contributes significantly in solving hidden hunger crisis especially among children and women in SSA (Okonya and Maass, 2014). Results revealed that cowpea leaves are a rich source of minerals such as iron, potassium, calcium, magnesium, phosphorus, manganese and zinc (Table 2). The mineral contents in cowpeas in this study was in the same range with some of the green leafy vegetables, including *C. gynandra* (Schönfeldt and Pretorius, 2011; Okonya and Maass, 2014; Kim et al., 2014; Omondi et al., 2017). However fluctuations can occur due to changes in production conditions, temperature and light just as seen in the carbohydrates dynamics. The dynamics of mineral concentrations in cowpea leaves during development was specific for different elements (Table 2). During cowpeas developments, most mineral contents such as zinc, potassium, phosphorous, nitrogen, magenesium and copper were observed to be stable although slight variations were observed. However, calcium decreased with age while Fe and Mn increased (Table 2). Previous studies have reported a wide variability of minerals in different cowpeas cultivars (Kim et al., 2014). However, little information is available about dynamics of mineral elements during the development of leafy vegetables.

5. Conclusion

Results from this study show the dynamics of phytonutrient composition in cowpea leaves based on developmental stages and postharvest storage conditions and duration. This raises the importance of biochemical markers such as chlorophyll, antioxidants, and carbohydrate contents amongst others in determination of harvesting indices for cowpeas, a nutrient-rich crop. Generally, the results indicated that

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

60−90 d as the maturity stage with optimal nutritional content, important for the human diet. Therefore, harvesting cowpeas when the nutrients are optimal can offer maximum benefits in terms of nutritional quality and also may compensate for losses incurred under suboptimal storage conditions. An optimal harvesting time would be important for the postharvest processing treatments, such as fermentation, solar drying or sun drying among others, and phytonutrients should also be analyzed after such treatments. Most phytonutrients in cowpeas would be preserved at cold storage at least up to four days. Although cold storage facilities are not available to many resource-poor farmers in SSA, use of on-farm evaporative coolers should be emphasized to preserve most of the beneficial phytonutrients in cowpea leaves and a cooling chain is strongly recommended if cowpea leaves need to be transported to supermarket/marketing centers.

Declarations

Author contribution statement

Dinah Kirigia: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Traud Winkelmann, Remmy Kasili: Conceived and designed the experiments; Wrote the paper.

Heiko Mibus: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the German federal ministry of education and research (BMBF) in collaboration with the ministry of economic cooperation and development (HORTINLEA, grant number 031A248E).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We acknowledge the HORTINLEA project, and the collaborating universities for the provision of space, materials and resources for this research, Hochschule

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Geisenheim University, Leibniz University Hannover and Jomo Kenyatta University, Kenya. Finally, we thank the WorldVeg Arusha, Tanzania, more specifically Dr. Fekadu Dinssa, for providing the cowpeas Tumaini variety seed which was used in this research.

References

Acedo, A.J.L., 2010. Postharvest Technology for Leafy Vegetables. https://doi.org/AVRDC-ADB Postharvest%20Projects%20RETA%206208/6376. AVRDC Publication No. 10−733.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2, 875−877.

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6, 1720−1731.

Avanza, M., Acevedo, B., Chaves, M., Añón, M., 2013. Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: principal component analysis. LWT - Food Sci. Technol. 51, 148−157.

Bergqust, S.Å., Gertsson, U.E., Knuthsen, P., Olsson, M.E., 2005. Flavonoids in baby spinach (Spinacia oleracea L.): changes during plant growth and storage. J. Agric. Food Chem. 53, 9459−9464.

Brabcová, M., Rychlovský, P., Němcová, I., 2003. Determination of nitrites, nitrates, and their mixtures using flow injection analysis with spectrophotometric detection. Anal. Lett. 36, 2303−2316.

Chipurura, B., 2010. Nutritional Content, Phenolic Compounds Composition and Antioxidant Activities of Selected Indigenous Vegetables of Zimbabwe. Thesis. Faculty of Science, Zimbabwe University. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.826.6609&rep=rep1&type=pdf.

Eghdami, A., Sadeghi, F., 2010. Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium*. Org. Chem. J. 2, 81−84. http://ocj.iau-saveh.ac.ir/Files/Journal/2012-01-17_02.02.14_9-eghdami.pdf.

Eveland, A.L., Jackson, D.P., 2012. Sugars, signalling, and plant development. J. Exp. Bot. 63, 3367−3377.

Ghasemzadeh, A., Nasiri, A., Jaafar, H.Z.E., Baghdadi, A., Ahmad, I., 2014. Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass (Clinacanthus nutans L.) in relation to plant age. Molecules 19, 17632−17648.

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Gomez, L., Bancel, D., Rubio, E., Vercambre, G., 2007. The microplate reader: an efficient tool for the separate enzymatic analysis of sugars in plant tissues − validation of a micro-method. J. Sci. Food Agric. 87, 1893−1905.

Goncalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E.A.S., Ferreira, L., Rodrigues, M., 2016. Cowpea (Vigna unguiculata L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. J. Sci. Food Agric. 96, 2941−2951.

Granot, D., Kelly, G., Stein, O., David-Schwartz, R., 2014. Substantial roles of hexokinase and fructokinase in the effects of sugars on plant physiology and development. J. Exp. Bot. 65, 809−819.

Grivetti, L.E., Ogle, B.M., 2017. Value of traditional foods in meeting macro-and micronutrient needs: the wild plant connection. Nutr. Res. Rev. 13, 31−46.

Grotewold, E., 2005. Plant metabolic diversity: a regulatory perspective. Trends Plant Sci. 10, 57−62.

Ibrahim, S.S., Habiba, R.A., Shatta, A.A., Embaby, H.E., 2002. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. Nahrung-Food 46, 92−95.

Jones Jr., J.B., 2001. Laboratory guide for conducting soil tests and plant analysis. In: Laboratory Guide for Conducting Soil Tests and Plant Analysis.

Kim, D., Kim, Y., Chon, S., Rim, Y., Choi, J., Kwon, O., 2014. Growth response and nutrient content of cowpea sprouts based on growth temperature and genetic resources. Kor. J. Crop Sci. 59, 332−340. https://www.cabdirect.org/cabdirect/FullTextPDF/2014/20143373645.pdf.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., Durand, M., 2013. Source-to-sink transport of sugar and regulation by environmental factors. Front. Plant Sci. 4 (272).

Lichtenthaler, H.K., 1987. Plant Cell Membranes, Methods in Enzymology. Elsevier.

Limantara, L., Martin Dettlinga, B., Indrawati, Renny, Indriatmokoa, Brotosudarmoa, Tatas Hardo Panintingjati, 2015. Analysis on the chlorophyll content of commercial green leafy vegetables. Proc. Chem. 225−231. Elsevier.

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Linguya, K.S., Moraa, O.C., Wangai, K.J., Chao, K.D., 2015. Potential of intercropping for management of some arthropod and nematode pests of leafy vegetables in Kenya. J. Agric. Sci. 60, 301−314.

Mathiventhan, U., Sivakanesan, R., 2013. Total phenolic content and total antioxidant activity of sixteen commonly consumed green leafy vegetables stored under different conditions. Eur. Int. J. Sci. Technol. 2, 123−132. https://www.researchgate.net/publication/263968117.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V., Milner, A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84, 407−412.

Modi, A.T., 2007. Growth temperature and plant age influence on nutritional quality of Amaranthus leaves and seed germination capacity. Afr. J. 33, 369−376. Online. http://www.wrc.org.za/Knowledge%20Hub%20Documents/Water%20SA%20Journals/Manuscripts/2007/05/WaterSA_2007_05_Article%209.pdf.

Moyo, M., Amoo, S.O., Ncube, B., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2013. Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. South Afr. J. Bot.

Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J., Schreiner, M., 2017. Indigenous leafy vegetables of Eastern Africa — a source of extraordinary secondary plant metabolites. Food Res. Int.

Nijveldt, R.J., Van Nood, E., Van Hoorn, D.E.C., Boelens, P.G., Van Norren, K., Van Leeuwen, P.A.M., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr.

Okonya, J., Maass, B., 2014. Protein and Iron composition of cowpea leaves: an evaluation of six cowpea varieties grown in eastern Africa. Afr. J. Food Nutr. Sci. Agric. Nutr. Dev. 14, 2129−2140.

Oloyede, F.M., Oloyede, F.A., Obuotor, E.M., 2013. Effect of plant maturity on the antioxidant profile of Amaranthus cruentus L. and Celosia argentea L. Bull. Environ. Pharmacol. Life Sci. 2, 18−21. Online. http://www.bepls.com/jan_2013/5.pdf.

Omondi, E.O., Engels, C., Nambafu, G., Schreiner, M., Neugart, S., Abukutsa-Onyango, M., Winkelmann, T., 2017. Nutritional compound analysis and morphological characterization of spider plant (Cleome gynandra) - an African indigenous leafy vegetable. Food Res. Int.

Saidi, M., Itulya, F.M., Aguyoh, J.N., 2010. Effects of cowpea leaf harvesting initiation time and frequency on tissue nitrogen content and productivity of a

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

dual-purpose cowpea − maize intercrop. HortScience 45, 369−375. http://hortsci.ashspublications.org/content/45/3/369.full.pdf.

Schönfeldt, H.C., Pretorius, B., 2011. The nutrient content of five traditional South African dark green leafy vegetables-A preliminary study. J. Food Compos. Anal. 24, 1141−1146.

Smith, A.M., Zeeman, S.C., Smith, S.M., 2005. Starch degradation. Annu. Rev. Plant Biol. 56, 73−98.

Spinardi, A., Ferrante, A., Spinardi, A., Ferrante, A., 2016. Effect of storage temperature on quality changes of minimally processed baby lettuce. J. Food Agric. Environ. 10 (1), 38−42.

Tyrakowska, B., Soffers, A.E.M.F., Szymusiak, H., Boeren, S., Boersma, M.G., Lemańska, K., Vervoort, J., Rietjens, I.M.C.M., 1999. TEAC antioxidant activity of 4-hydroxybenzoates. Free Radic. Biol. Med. 27, 1427−1436.

Veitch, N.C., 2007. Isoflavonoids of the leguminosae. Nat. Prod. Rep. 24, 417−464.

Wafula, E.N., Franz, C.M.A.P., Rohn, S., Huch, M., Mathara, J.M., Trierweiler, B., 2016. Fermentation of african indigenous leafy vegetables to lower post-harvest losses, maintain quality and increase product safety. Afr. J. Hortic. Sci. 9. http://hakenya.net/ajhs/index.php/ajhs/article/view/151/116.

Wojakowska, A., Muth, D., Narozna, D., Madrzak, C., Stobiecki, M., Kachlicki, P., 2013. Changes of phenolic secondary metabolite profiles in the reaction of narrow leaf lupin (Lupinus angustifolius) plants to infections with Colletotrichum lupini fungus or treatment with its toxin. Metabolomics 9, 575−589.

Yamauchi, N., Watada, A.E., 1991. Regulated chlorophyll degradation in spinach leaves during storage. J. Am. Soc. Hortic. Sci. 116 (1), 58−62. http://journal.ashspublications.org/content/116/1/58.full.pdf+html.

Zhao, D., MacKown, C.T., Starks, P.J., Kindiger, B.K., 2010. Rapid analysis of nonstructural carbohydrate components in grass forage using microplate enzymatic assays. Crop Sci. 50, 1537−1545.

https://doi.org/10.1016/j.heliyon.2018.e00656
2405-8440/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).