Effect of Different Packaging Materials on β-carotene and Vitamin C Content of Minimally Processed African Indigenous Leafy Vegetables

Grace Cheptoo[1], Willis Owino[1*] and Glaston Kenji[1]

[1]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

***Corresponding author:** willis@agr.jkuat.ac.ke

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ABSTRACT

African Indigenous Vegetables (AIVs) are highly perishable and susceptible to high postharvest losses. This study aimed at examining the retention of β-carotene and vitamin C content of blanched and unblanched vegetable amaranth, African nightshade and stinging nettle, subjected to Ziploc® and Xtend® bag packaging under freezing and room temperature conditions. Both mature and young fresh amaranth leaves had the highest β-carotene content at 75.08±1.3 mg/100g and 44.53±3.1 respectively. African Nightshade had the lowest β-carotene content of 45.05±1.9 and 37.12±3.0 mg/100g respectively. Stinging nettle exhibited the highest Vitamin C content at 197.75±6.9 and 149.7±6.1 mg/100g at both the young and mature stages respectively. Blanching had a significant difference (P<0.05) on both β-carotene and vitamin C retention as the contents in blanched samples were lower by about 10-15% as compared to fresh leaves samples. Under Ziploc® bag packaging in frozen temperatures, both young and mature amaranth leaves were able to retain a significantly higher (at least 50%) β-carotene content by week 4, with the highest retention being observed in unblanched samples. Blanching of young African nightshade and stinging nettle leaves led to least retention of β-carotene by week 4 (~30%). However, in all the AIVs, the retention of Vitamin C was lower with less than 50% of the initial Vitamin C content retained by week 4. The AIVs were able to keep their marketability for 5-7 days in Xtend® bag packaging under room conditions. However, nutrient loss was quite high with fresh amaranth losing over 80% of its initial β-carotene and vitamin C content. This study indicates that modified atmosphere packaging in Ziploc® bag and Xtend® bags, stage of maturity and blanching, had significant effect on the on the stability of β-carotene and vitamin C of the AIVs.

INTRODUCTION

African Indigenous Vegetables (AIVs) are gaining prominence as excellent source of pro-vitamin A, C, iron as well as protein, minerals and fiber. In addition, AIVs are rich in antioxidants and other health related phytochemicals (Kamga et al., 2013; Orech et al., 2005). Over the past 15 years, AIVs have seen significant growth in demand and market (Aura, 2013). High postharvest losses are experienced during production, harvesting, transport and retailing due to lack of adequate capacity to maintain cold chains in these AIVs (Habwe, 2008). In addition, after harvesting, large portions are lost because of poor handling and marketing conditions. Postharvest losses in AIVs can be as high as 50% (Aseno-Okyere, 2012).

Traditional postharvest methods such as sun drying, solar drying and fermentation, are still used by most farmers and other stakeholders in the AIV value chain to reduce losses (Stoilova et al., 2015). However, there have been changes in market trends and demands from consumers, which have spurred progress in structural transformation to modern systems of postharvest technology. Modified atmospheres (MA) packaging is commonly used to extend the shelf life of fruits and vegetables, through reduction in respiration rate and inhibiting or delaying enzymatic reactions in the commodity (Oloo, 2010). Improved MA packaging material such as Xtend® and Ziploc® have been introduced onto the marketplace to protect fresh produce from contamination and spoilage as well as to maintain the nutritional quality and enhance sensory value of commodity (Seevaratnam et al., 2012; Zenoozian, 2011).

Minimal processing (such as blanching) involves subjecting the vegetables to different processing steps to obtain an edible product that offers fresh-like qualities expected by the consumer. Blanching process may affect the color, texture, flavor, and nutritional quality of the vegetable. The objective of this study was to determine the retention of β-carotene and vitamin C contents of blanched and unblanched vegetable amaranth *(Amaranthus dubious*), African nightshade *(Solanum scabrum)* and stinging nettle (*Urtica dioica*), subjected to MA packaging in Ziploc® and Xtend® bag under specific temperature conditions ideal for the two packaging bags. The AIVs amaranth and African nightshade, were selected because of their higher consumption and availability in the market whereas stinging nettle was selected for comparison due to its traditional consumption as a vegetable.

MATERIAL AND METHODS

Plant material. Seeds of Amaranth (*Amaranthus dubious*) and African nightshade *(Solanum scabrum*) were purchased from Simlaw Seeds Company, Kenya. They were grown in a randomized block design at Jomo Kenyatta University of Agriculture and Technology (Juja, Kenya), experimental research farm. Plots were divided into thirds, having a size of 5m by 4m with each having 3 replicates. Ploughing was done twice and land well leveled. Seeds were directly sown into furrows at an inter-row spacing of 40 cm. During planting, compost manure was sprinkled at the rate of 10 tonnes/hectare. Irrigation to the crops was done daily as well as weeding and hoeing. Planting was done during the month of 2nd December 2016 – 23rd February 2017. Two weeks after germination, the plants were thinned to a spacing of 15 cm between the plants. Stinging nettle (*Urtica dioica*) was sourced from a commercial farmer in Juja farm.

Harvesting and sample preparation. Leaves of the AlVs were harvested at two stages: a young stage (5 weeks) and mature stage (10 weeks) after planting. After harvesting, the leaves were washed with running water, and allowed to drain excess water, then divided into two portions, blanched and unblanched fresh leaf samples. Blanching was done using hot water at 95±1°C for 30 seconds with the ratio of 1:7 vegetables to water (g/ml) following the method by Tanongkankit *et al.* (2010). The blanched samples were removed and quickly dipped in ice-cold water (4°C) to stop enzymatic activity. The blanched samples were then left in a wire mesh for water to drain.

Packaging. The samples were packaged in two different materials: Ziploc® bags and Xtend® bags in order to determine the nutritional stability (β-carotene and vitamin C) of the minimally processed ALVs. The Xtend® bags designed for the storage of herbs and vegetables under room temperature conditions were obtained from Amiran Kenya. The Xtend® bags are MA bags characterized by high moisture vapor transmission rates in comparison to polyethylene films. This assures that excess moisture is eliminated, in the event that condensation forms within the bag. Fresh and blanched leaf samples were divided into two batches. One of the batches were packed in a Ziploc® bags and sealed. It was then frozen in a freezing chamber at (-18°C) while the other batch was packed in Xtend® bags and then stored at room temperature (25°C). The samples were then analyzed for β-carotene and vitamin C.

Determination of β-carotene. β-carotene content was analyzed spectrophotometrically as described by Rodriquez-Amaya and Kimura (2004). Two grams of AIV fresh samples were weighed and ground thoroughly in a motor and pestle using acetone. The acetone extract was then transferred to volumetric flask and the residue extracted again with acetone. This was repeated until the residue no longer gave (orange) color to acetone. Twenty-five mL of the extract was evaporated to dryness on a rotary vacuum evaporator and the residue dissolved in petroleum ether. The solution was introduced into open chromatographic column packed with silica gel, eluted with petroleum ether and collected up to 25mL. The absorbance of the solution was determined at 440nm using UV-vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) and plotted against their corresponding standard concentrations.

Determination of Vitamin C content. Vitamin C content was determined using High Performance Liquid Chromatography (HPLC) (Vikram *et al*., 2005). Five grams of the sample was weighed and extracted with 0.8% metaphosphoric acid. The extract was then made to 20 mL and centrifuged at 10000 rpm for 10 minutes. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. This was then filtered using cotton wool and micro-filtered through 0.45 μ filter and 20μL injected into the HPLC. HPLC analysis was done using Shimadzu (10A model; Tokyo, Japan) and a UV-Vis detector. The mobile phase was 0.8% metaphosphoric acid, at 1.1 mL/min flow rate and wavelength of 266.0 nm.

Statistical analysis. Data were subjected to analysis of variance using Stata version 12 software (Stata Corp.) while Duncan's test at 0.05 significance level were used to perform mean variation.

RESULTS

Effect of Ziploc bag packaging on β-carotene content (mg/100g) of ALVs. In all the AIVs, the mature leaves had significantly higher ($P<0.05$) β-carotene content as compared to the young leaves (Figs. 1-6). The fresh amaranth had the highest β-carotene content levels (44.53±3.1 and 75.08±1.4 mg/100g) both at young and mature stages (Fig. 1). After blanching, on Day 1, the β-carotene content of the leaves was 36.22±2.2 and 68.98±3.8mg/100g at young stage and mature stages, respectively. Samples were then packaged in Ziploc® bags and stored under frozen conditions. Over time from week 1 to week 4, there was a gradual decline in β-carotene in all samples. However, the mature leaves maintained higher contents β-carotene as compared to the young leaves. By week 4, mature amaranth leaf samples stored in Ziploc® bags under frozen condition, maintained the highest β-carotene content at 55.05±1.23 mg/100g, blanched mature leaf samples had 39.42±2.0 mg/100g while the young fresh and the unblanched leaf samples maintained value of 33.05±2.0 and 20.64±2.0 mg/100g respectively (Fig. 1).

The mature African nightshade leaves had a β-carotene content of 45.05±1.9 mg/100g that dropped to 27.05±1.5 mg/100g by week four (Fig. 2). Blanching of the mature African nightshade leaves led to a significant loss in β-carotene content under frozen storage in Ziploc® bags, from 40.5±0.9 to 12.6±1.2 mg/100g. The young unblanched African nightshade leaves maintained a relatively higher β-carotene content throught out the storage period compared to blanching of mature leaves (Fig. 2). Mature stinging nettle leaves similarly had higher β-carotene content (61.64±1.8) with blanching leading to some loss on day 1(55.62±2.3) (Fig. 3). The β-carotene content of young stinging nettle leaves was 39.17±1.4 mg/100g. In all four samples, the β-carotene content gradually declined under frozen conditions in Ziploc® bags.

Effect of Xtend® bag packaging on β-carotene content (mg/100g) of AIVs. The Xtend® bags combines the effect**s** of MA, modified humidity and condensation control to prolong the shelf life of perishable commodities even under normal room temperatures. The Xtend® bag packaging was able to extend the marketability of unblanched Amaranth leaves to day 7 for both mature and young samples. The β-carotene content declined from 75.53±1.3 to 10.85±2.5 and 44.43±1.1 to 4.95±1.8 in mature and young leaves respectively (Fig. 4). Blanching of the leaves drastically reduced the β-carotene but also their marketability which deteriorated by day 3 for both young and mature leaves. Packaging **u**nblanched African nightshade in Xtend® bags extended their marketability to day 5 with β-carotene content declining from 45.05±1.2 mg/100g to 25.16±1.0 mg/100g and 37.13±2.9 mg/100g to 18.77±0.6 mg/100g in both mature and young leaves respectively (Fig. 5). Blanching and storage of the leaves shortened their marketability such that they had deteriorated by day 3 with β-carotene dropping to 23.77±0.**5** mg/100g and 19.33±1.01 mg/100g in the mature and young leaves. The Xtend® bag packaging under room conditions led to a reduction in β-carotene content and marketability of blanched stinging nettle leaves by day 3 (Fig. 6). The β-carotene content dropped from 61.65±2.4 mg/100g to 37.94±1.5mg/100g and 31.18±1.4 mg/100g to 16.73±1.4 mg/100g in mature and young stinging nettle leaves. The nonblanched leaves extended the marketability to day 5 and the young immature leaves accumulated only about half the β-carotene content compared to of the mature stinging nettle leaves.

Effect of storage (Ziploc® bag) on Vitamin C content. The Ziploc® bag packaging under frozen conditions maintained significant ($P<0.05$) levels of vitamin C contents of amaranth from day 1 till week 4, with a gradual decline in all the samples (Fig. 7). Similar to β-carotene content, the fresh mature amaranth leaves had higher contents of vitamin C (171.69±4.0 mg/100g) as compared to the young leaves (99.10±4.4). Blanching reduced the level of Vitamin C in both mature and young leaves throughout the storage period. The African nightshade had a Vitamin C content of 189.1±9.2 mg/100g compared to the young leaves at 127.8±8.0 mg/100g (Fig. 8). Blanching reduced the Vitamin C to 173.02±7.6 mg/100g and 110.24±8.0 mg/100g for mature and young leaves respectively followed by a gradual decline. Fresh stinging nettle at the young stage and mature stage had the highest content of vitamin C of 149.71±6.13 and 197.75±5.88 mg/100g compared to blanched (Fig. 9). With packaging and storage, fresh mature stinging nettle maintained the highest content of vitamin C by week 4 at 101.5±6.18 (Fig. 9). In all cases blanching retained vitamin C content but was still less than fresh and over time with and without packaging there is a gradual loss of vitamin C.

Effect of packaging (Xtend® bag) on Vitamin C. Under Xtend**®** bag packaging at room temperature fresh amaranth samples had significantly higher ($P<0.05$) vitamin C content than the blanched samples for both mature and young leaves (Figure 10). Blanching reduced the vitamin C content (from 99.11±4.4 to 85.05±6.7 and 171.60±8.0 160.14±2.5 mg/100g) at young and mature stages on day 1 (Fig. 10). The vitamin C content declined by day 3 in blanched samples, but a significant amount was detectable by day 5 in the fresh samples.

The Xtend**®** bag packaging reduced the Vitamin C content of mature African nightshade from 189.1±9.2 mg/100g to 79.1±7.2 compared to the young leaves which reduced from a 127.8±8.0 mg/100g to 61.65±4.9 (Fig. 11). Blanching reduced the Vitamin C content to 173.02±7.6 mg/100g and 110.24±8.0 mg/100g for mature and young leaves respectively followed by a rapid decline by day 3. Blanched stinging nettle exhibited the highest content (123.65±2.5 and 183.12±2.2 mg/100g) at both stages of maturity on Day 1(Fig. 12). At Day 5, unblanched fresh stinging nettle in Xtend**®** bag packaging retained the highest content of vitamin C of 63.05±2.2 mg mg/100g and 97.8±5.9 mg/100g at young stage and mature stages respectively (Fig. 12).

DISCUSSION

Due to their high perishability, AIVs require a high humidity environment to avoid nutritional losses that accompany excessive wilting and lead to postharvest losses. MA packaging has the benefits of retarding the nutritional degradation that accompanies the senescence processes, in addition to prevention of water loss and wilting in vegetables. This study compared the nutritional retention of specific AIVS harvested at different stages under Ziploc® and Xtend**®** bag packaging under freezing and room temperature conditions. Maturation in vegetables has been reported to be accompanied by increased carotenogenesis (Arscott, 2013). We also found that β-carotene content was significantly higher ($P<0.05$) in mature leaves than in younger leaves in each of the AIVs.

Each of the AIVs evaluated in this study showed their inherent unique nutritional composition evidenced by the varying degree of β-carotene and vitamin C contents. Both mature and young fresh amaranth leaves had the highest β-carotene content at 75.08±1.3 mg/100g and 44.53±3.1 respectively. The African Nightshade had the lowest β-carotene content of 45.05±1.9 and 37.12±2.9 mg/100g respectively. In contrast, stinging nettle had the highest Vitamin C content (197.75±6.9 and 149.7±6.1 mg/100g at both the young and mature stages respectively). A similar pattern was observed in Vitamin C that was significantly higher ($P<0.05$) in mature leaves as compared to young leaves. This agrees with Yamada et al. (2003) who reported that mature spinach leaves had a higher content of vitamin C than at a younger stage. Vitamin C content in broccoli leaves were also reported to increased with maturation (Omary *et al*., 2003).

Minimal processing such as blanching is known to alter the physical matrix of the vegetable by generally disrupting the leaf tissue cells making the organelles containing carotenoids be more accessible (Bédouet *et al*., 2015). In this study, blanching had a significant difference ($P<0.05$) on both β-carotene and vitamin C as the contents in blanched samples were lower by a range of 10-15% as compared to fresh leaves samples. Blanched spinach underwent a 70% in Vitamin C in comparison with raw spinach (Jaworska, 2005). In addition, Puupponen-Pimia (2003) reported losses during blanching/freezing of approximately 30% for peas, 10% for green beans, 30% for broccoli, and 40% for spinach in Vitamin C. Vitamin C is highly sensitive to oxidation and it is the least stable nutrient during processing and together with β-carotene may leach into blanching medium (Franke *et al*., 2004). After blanching and freezing peas, green beans and broccoli, a 30% loss in β-carotene content was observed (Bouzari *et al*., 2014).

The AIVs were packaged in Ziploc® and kept under frozen temperature up to 4 weeks to appraise the extent of nutrient retention with or without blanching. There was a gradual decline in both β-carotene and Vitamin C content. Both young and mature amaranth leaves were able to retain a significantly higher (at least half of their initial) β-carotene content by week 4, with the highest retention being observed in unblanched samples. Blanching of young African nightshade and stinging nettle lead to least retention of β-carotene by week 4 (~30%). However, with each of the AIVs, the retention of Vitamin C was lower as there was less than 50% of the initial Vitamin C content retained by week 4. Similar findings were reported by Prabhu and Barrett (2009) where 31% and 72 % of cassia tora leaves and 59% and 60% of *Corchorus tridens* loss of initial ascorbic acid during freeze storage for 14 days were observed. In addition, Hunter and Fletcher (2002) noted poor retention of vitamin C of spinach after storage. This could be attributed to isomerization and oxidation reaction during storage and blanching that affect the stability of β-carotene and Vitamin C (Schieber & Carle, 2005).

The AIVs were able to keep for 5-7 days under Xtend® bag in room conditions. However, as expected, the nutrient loss was high with fresh amaranth losing over 80% of its initial β-carotene and vitamin C content. Loss in Vitamin C content in amaranth leaves under MA packaging at room temperature for has previously been reported (Nyaura et al, 2014). However, African nightshade and stinging nettle retained about 50% of their initial β-carotene and vitamin C content by Day 5, which was the limit of marketability. Blanching reduced the marketability of African nightshade and stinging nettle to only 3 days. Similar results were reported by Anjum *et al.* (2008), where a reduction of 59.5% and 23.3%, in β-carotene content of lettuce and spinach stored at room temperature.

CONCLUSION

Use of MA packaging with Ziploc® bag and Xtend® bags, stage of maturity and blanching had significant effects on the stability of β-carotene and vitamin C in leaf amaranth, African nightshade and stinging nettle. Use of Ziploc® bags under frozen conditions retained nutrients for up to 4 weeks. However, this requires energy and its application in reducing postharvest losses of AIVs is subject to cost implications. The Xtend® bags under room conditions is a low-cost method that can retain the nutrient content and extend the shelf life of AIVs for between 5-7 days.

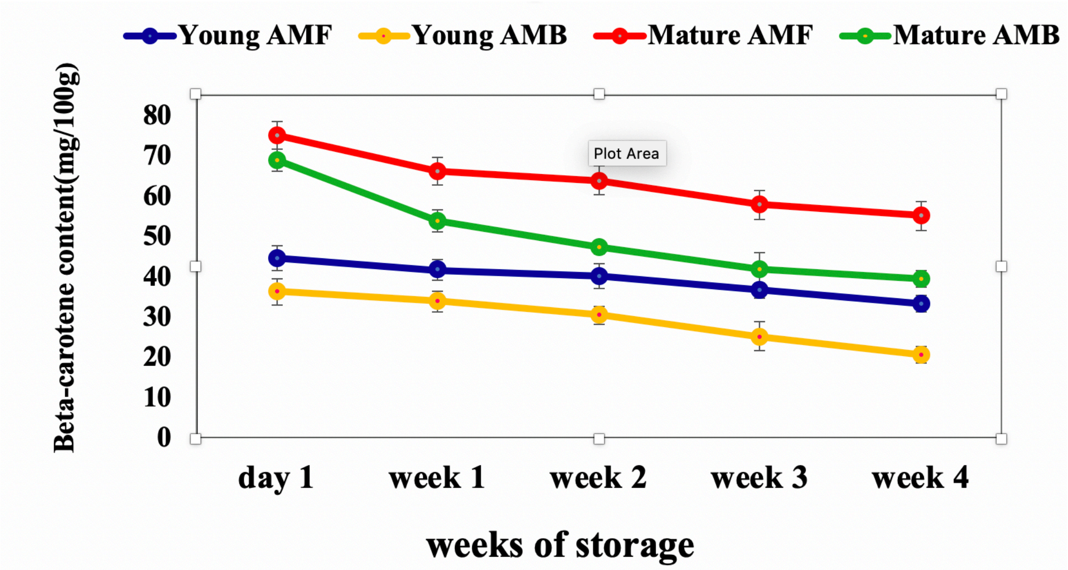

Figure 1: Effect of storage (Ziploc®) on β-carotene content (mg/100g) of frozen Amaranth at young and mature stage. Red color line represents mature amaranth fresh, green color line is mature blanched amaranth, blue color line represents young amaranth fresh and yellow color line is-young blanched amaranth. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). AMF-amaranth fresh, AMB-amaranth blanched.

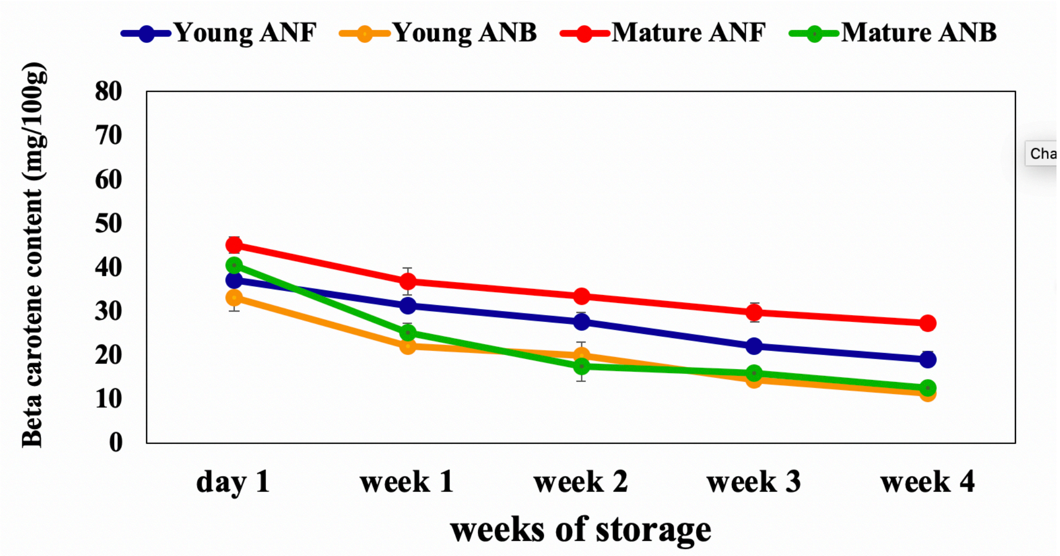

Figure 2: Effect of storage (Ziploc®) on β-carotene content (mg/100g) of frozen African Nightshade. Red color line represents mature fresh African Nightshade, green color line is mature blanched African Nightshade, blue color line represents young African Nightshade fresh and yellow color line is young blanched African Nightshade. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). ANF-African nightshade fresh, ANB-African nightshade blanched.

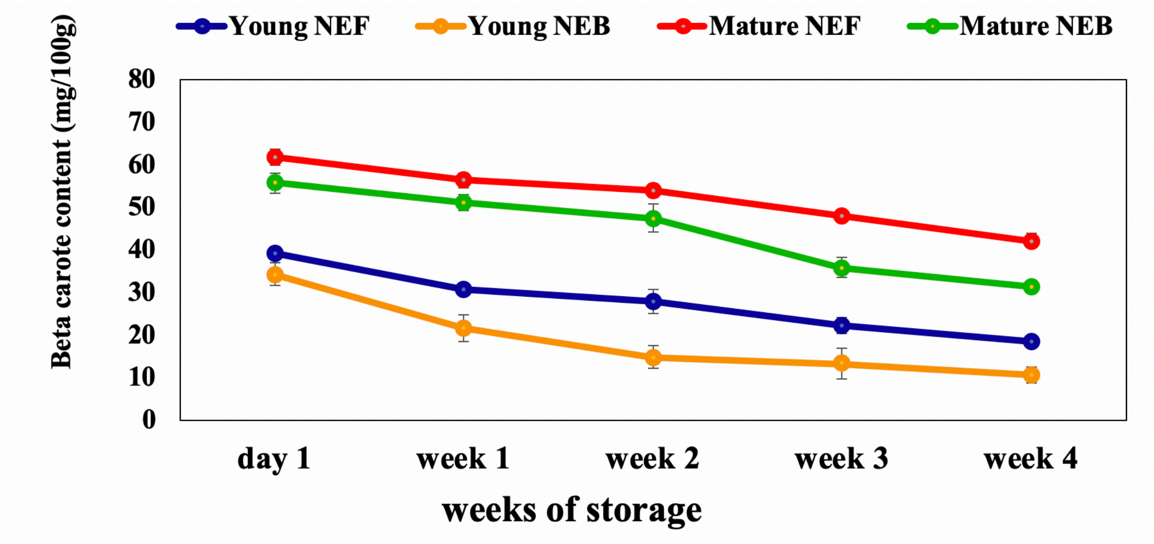

Figure 3: Effect of storage (Ziploc®) on β-carotene content (mg/100g) of frozen stinging nettle. Red color line represents mature fresh stinging nettle, green color line is mature blanched stinging nettle, blue color line represents young stinging nettle fresh and yellow color line is young blanched stinging nettle. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). NEF-Stinging nettle fresh, NEB-Stinging nettle blanched.

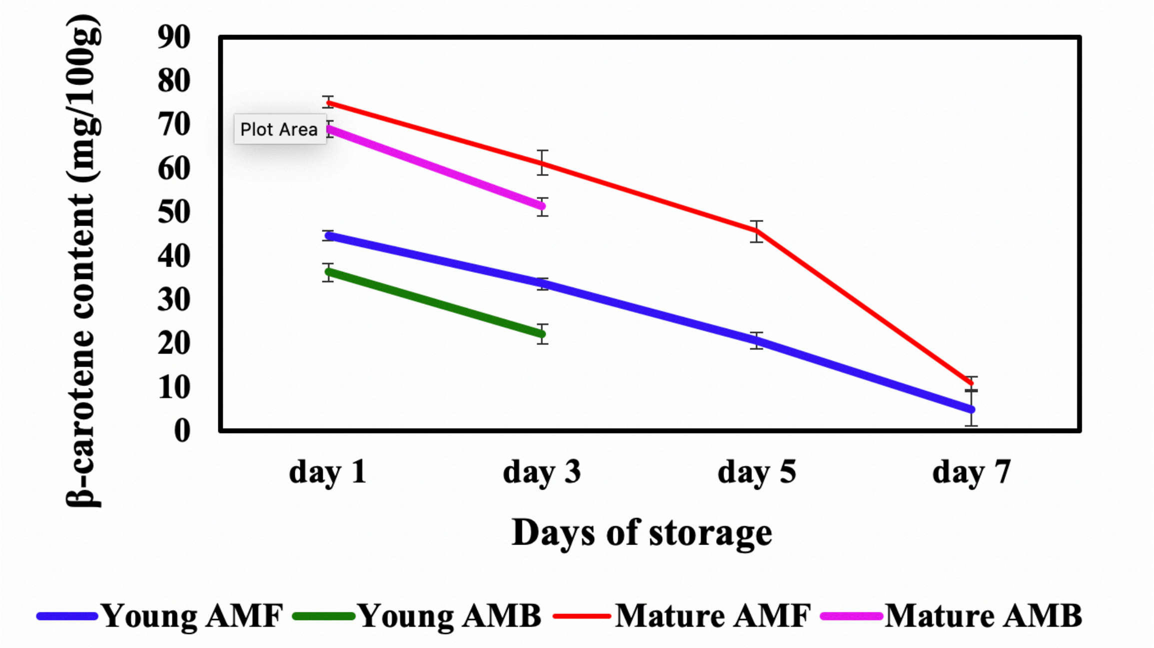

Figure 4: Effect of storage (Xtend® bag) on β-carotene of amaranth kept at room temperature. Red color line represents fresh mature amaranth, green color line represents young blanched amaranth, blue color line represents young fresh amaranth while purple color line is mature blanched amaranth. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). AMF-amaranth fresh, AMB-amaranth blanched.

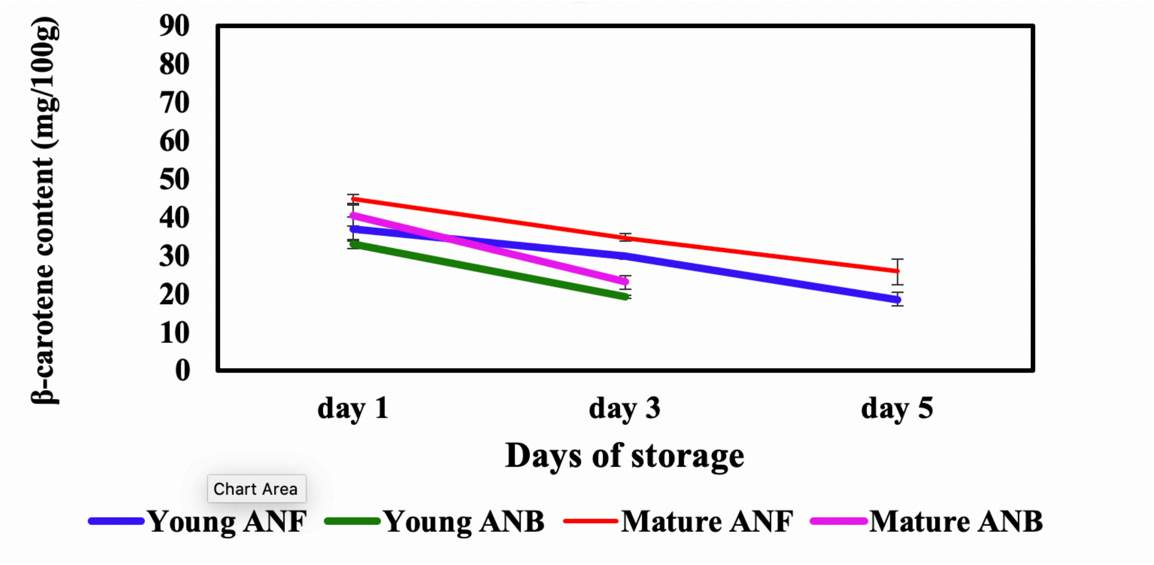

Figure 5: Effect of storage (Xtend® bag) on β-carotene of African nightshade kept at room temperature. Red color line represents fresh mature African nightshade, green color line represents young blanched African nightshade, blue color line represents young fresh African nightshade while purple color line is mature blanched African nightshade. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). ANF-African nightshade fresh ANB-African nightshade blanched.

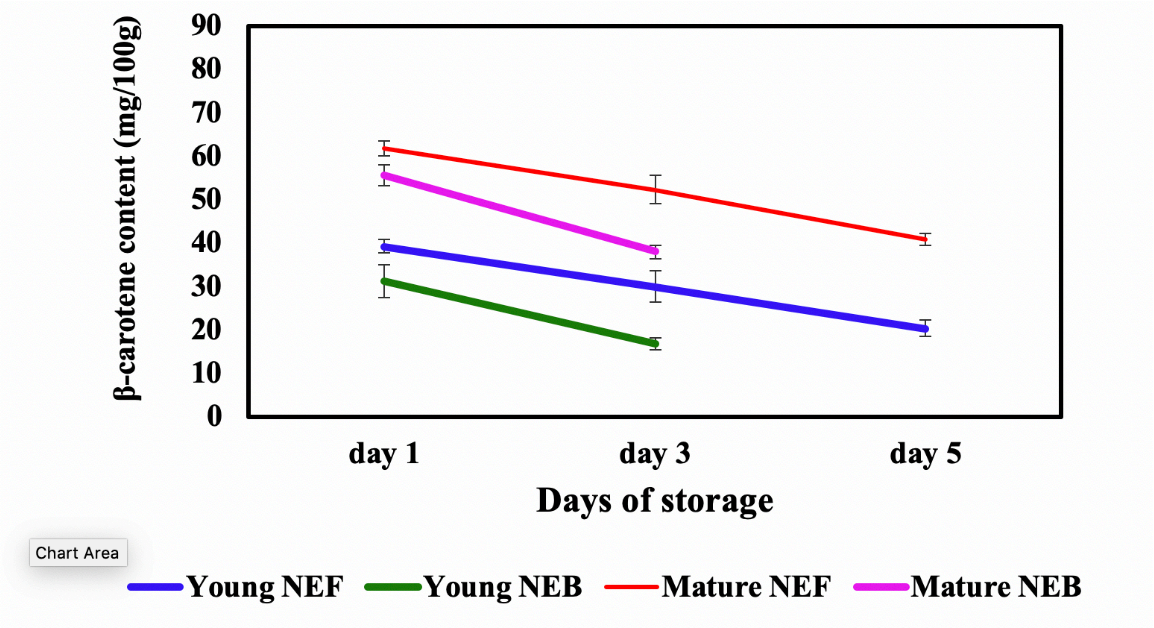

Figure 6: Effect of storage (Xtend® bag) on β-carotene of stinging nettle kept at room temperature. Red color line represents fresh mature stinging nettle, green color line represents young blanched stinging nettle, blue color line represents young fresh Stinging nettle while purple color line is mature blanched stinging nettle. Values are presented as means of three replicates (mg/100g) of three replicates (± SD). NEF-stinging nettle fresh, NEB-stinging nettle blanched.

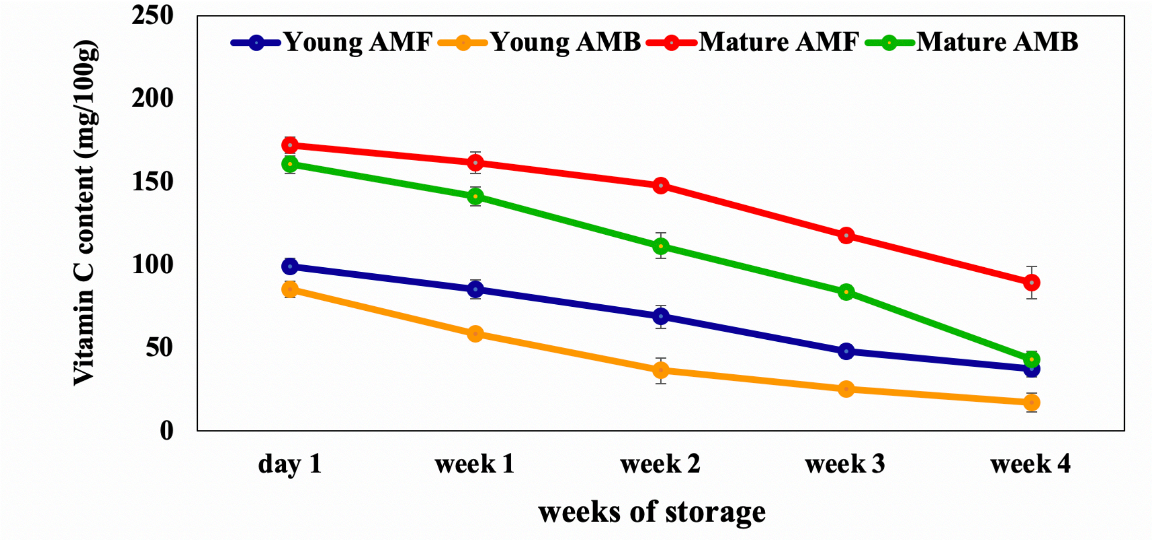

Figure 7: Effect of storage (Ziploc®) on Vitamin C content (mg/100g) of frozen Amaranth. Red color line represents mature fresh amaranth, green color line is mature blanched amaranth, blue color line represents young amaranth fresh and yellow color line is young blanched amaranth. Values are presented as means (mg/100g) of three replicates (± SD). AMF-amaranth fresh, AMB-amaranth blanched.

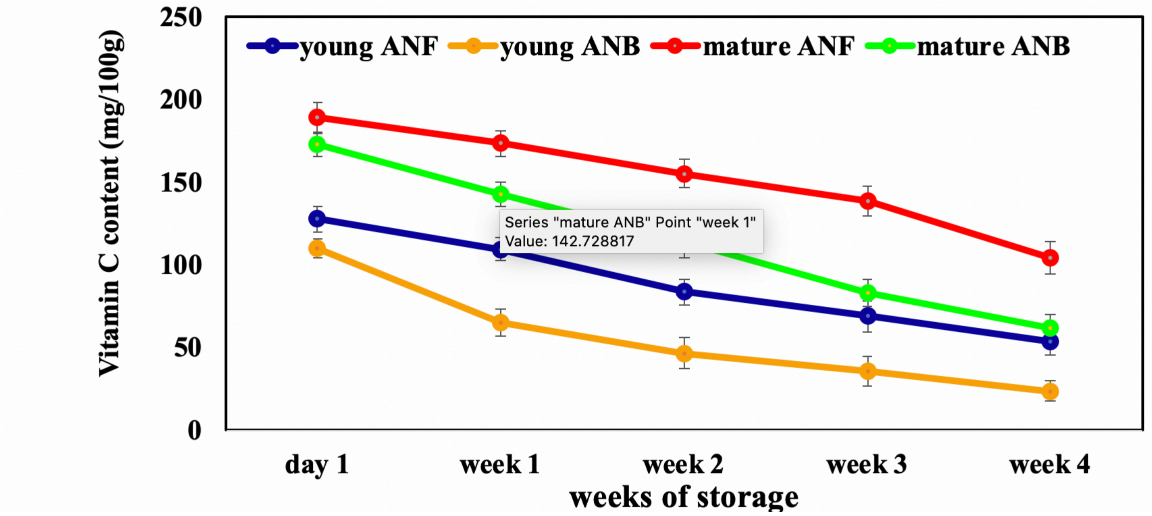

Figure 8: Effect of storage (Ziploc®) on vitamin C content (mg/100g) of frozen African nightshade. Red color line represents mature fresh African nightshade, green color line is mature blanched African nightshade, blue color line represents young African nightshade fresh and yellow color line is young blanched African nightshade. Values are presented as means (mg/100g) of three replicates (± SD). ANF-African nightshade fresh, ANB-African nightshade blanched.

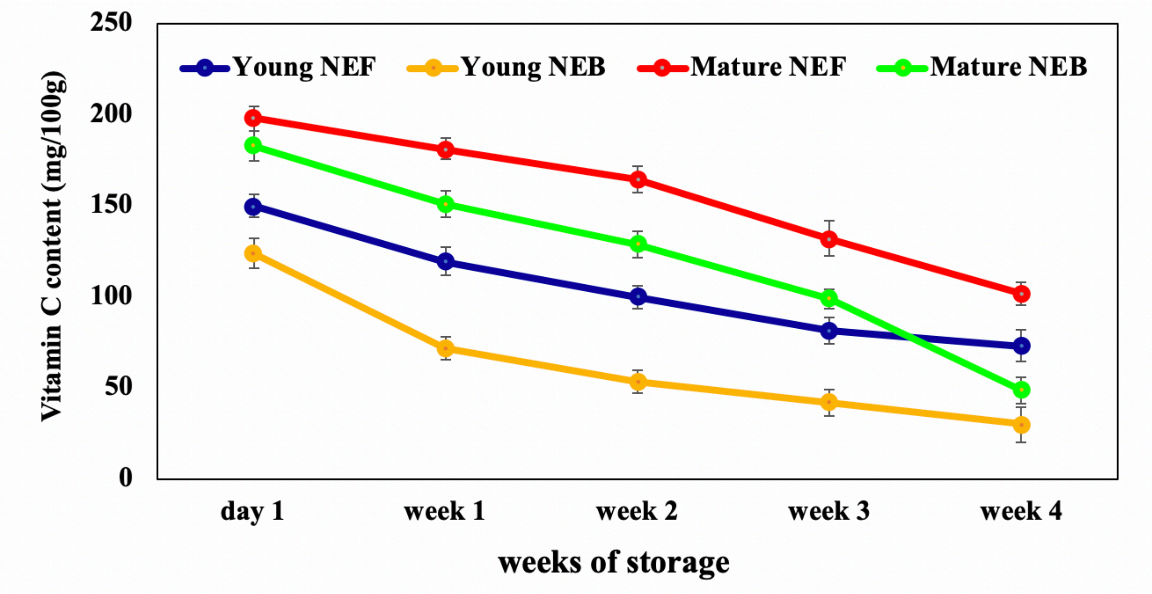

Figure 9: Effect of storage (Ziploc®) on vitamin C content (mg/100g) of frozen stinging nettle. Red color line represents mature fresh stinging nettle, green color line is mature blanched stinging nettle, blue color line represents young stinging nettle fresh and yellow color line is young blanched stinging nettle. Values are presented as means (mg/100g) of three replicates (± SD). NEF-Stinging nettle fresh, NEB-Stinging nettle blanched.

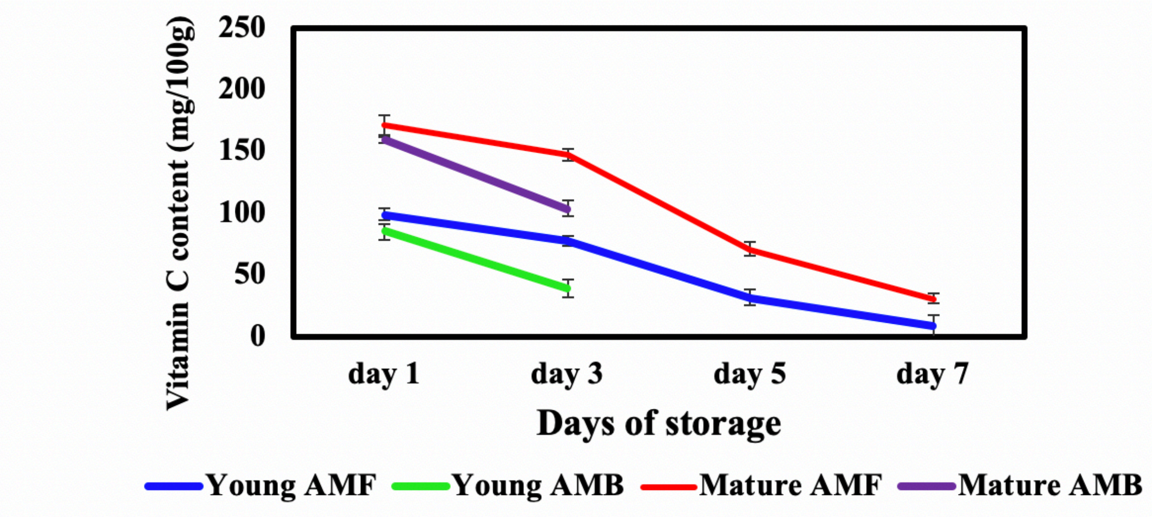

Figure 10: Effect of storage (Xtend®) on vitamin C content (mg/100g) of amaranth at room temperature. Red color line represents fresh mature amaranth, green color line represents young blanched amaranth, blue color line represents young fresh amaranth while purple color line is mature blanched amaranth. Values are presented as means (mg/100g) of three replicates (± SD). AMF-amaranth fresh, AMB-amaranth blanched.

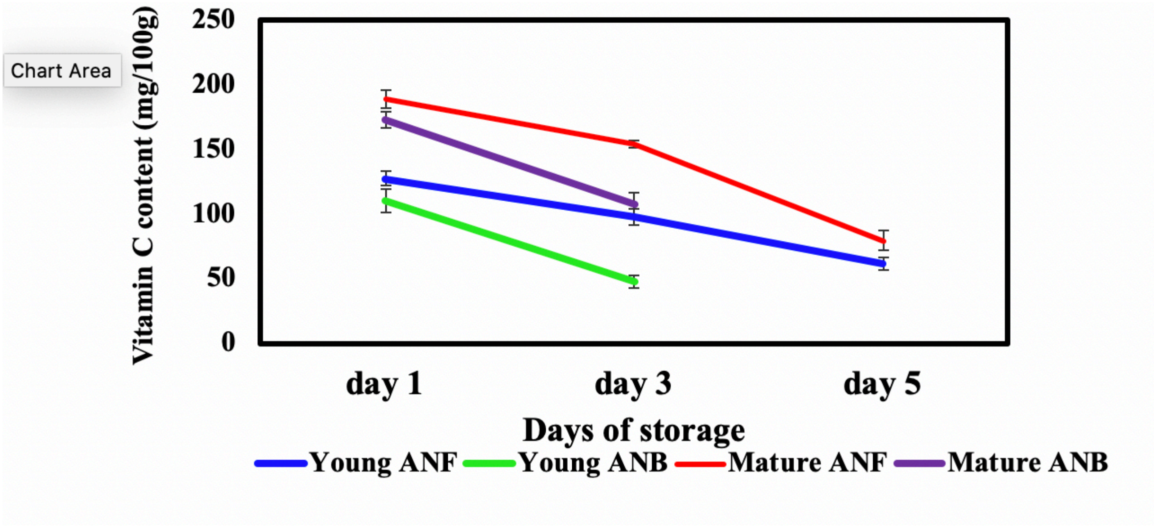

Figure 11: Effect of storage (Xtend®) on vitamin C content (mg/100g) of African nightshade at room temperature. Red color line represents fresh mature African nightshade, green color line represents young blanched African nightshade, blue color line represents young fresh African nightshade while purple color line is mature blanched African nightshade. Values are presented as means (mg/100g) of three replicates ± SD). ANF-African nightshade fresh, ANB-African nightshade blanched

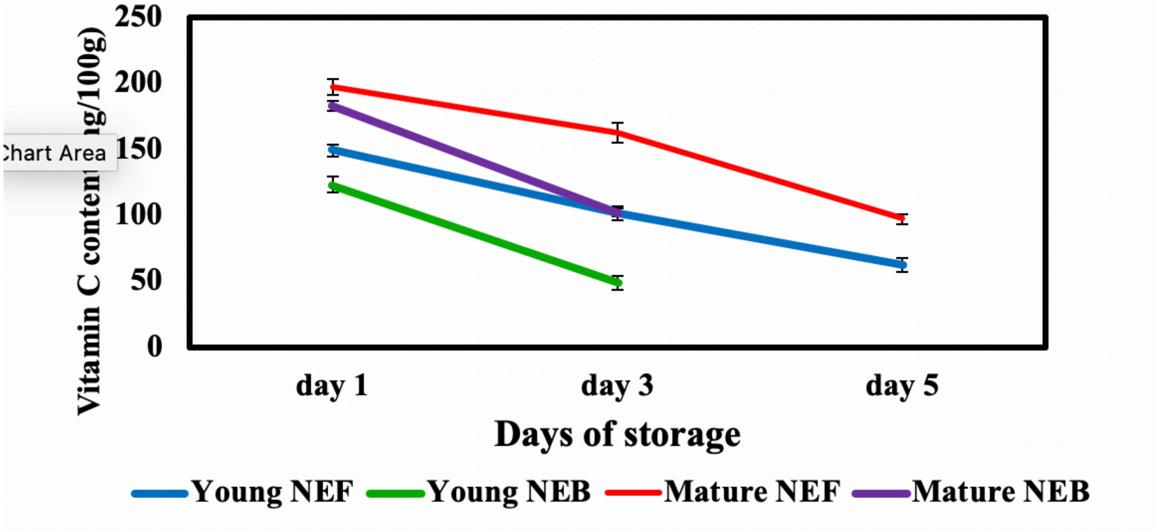

Figure 12: Effect of storage (Xtend®) on vitamin C content (mg/100g) of stinging nettle at room temperature. Red color line represents fresh mature stinging nettle, green color line represents young blanched stinging nettle, blue color line represents young fresh Stinging nettle while purple color line is mature blanched stinging nettle. Values are presented as means (mg/100g) of three replicates (± SD). NEF-Stinging nettle fresh, NEB-Stinging nettle blanched.

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