

Bioactive Components of Leafy Vegetable Edible Amaranth (*Amaranthus mangostanus* L.) as Affected by Home Cooking Manners

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Abstract The objective of the current study is to investigate how home cooking, a common way for many societies to prepare vegetables before consumption, affect bioactive components and antioxidant capacities of a commonly consumed leafy vegetable edible amaranth. The amaranth was cooked by simmering, boiling, frying, blanching and steaming. The contents of total phenolics, anthocyanins, *L*-ascorbic acid, carotenoids, lutein, beta carotene and ferric reducing antioxidant power (FRAP) of edible amaranth were determined after the cooking by colorimetric assays. Home cooking proved to degrade anthocyanins but increased carotenoids. Steaming increased total phenol content (TPC) about 50% while simmering reduced 31.1% of TPC. Simmering, frying and blanching deduced *L*-ascorbic acid content by 18.6%, 17.2%, and 14.0%, respectively. Steaming increased *L*-ascorbic acid by 21.7%. Both lutein and beta-carotene content was reduced by frying but increased by other methods. FRAP values of cooked vegetable were higher than the raw counterpart, which indicated the cooking increased the antioxidant capacities of the edible amaranth.

Keywords: *amaranth home cooking, vegetable antioxidants, amaranth carotenoids, amaranth phenolics*

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1. Introduction

It is widely accepted that vegetables play important roles in preventing the development of the cardiovascular diseases, aging-related diseases, obesity, cancers and improving human's memory (Wayne et al., 2000). The health promoting effects of vegetables is attributed to their natural dietary antioxidants. Dietary antioxidants prevent free radicals relative to aging such as reactive oxygen species in the human body (Nilsson et al., 2004). The free radical theory of aging involves cumulative damage as the of the natural free radical oxidative changes, which over time results in increasing antigenicity, protein changes, and oxidative DNA damages (Edelstein et al., 2009). Vitamin C is a powerful antioxidant contributing to normal function of the immune system (Melvin et al., 2010). Polyphenolic compounds have most antioxidant function acting as electron donors, electron acceptors, decomposer of peroxide and hydroperoxides, metal activators and deactivators and UV absorber (Svobadva et al., 2003). Anthocyanin is demonstrated powerful antioxidant properties against low- density lipoprotein oxidation to reduce the risk of the coronary heart disease (Wallace, 2011). The carotenoids are yellow to red pigment with major dietary representatives α -carotene, β -

carotene, lutein, cryptoxanthin, zeaxanthin and lycopene. The carotenoids are scavengers of reactive oxygen species (ROS) formed in physiological processes. ROS are able to damage biologically relevant molecules such as DNA, protein, lipids and carbohydrates (Kumpulainen et al., 1999).

Amaranth (*Amaranthus mangostanus* L.), commonly called 'Xiancai' in Chinese, is an annual herb in the family of Amaranthaceae. As a kind of vegetables, Amaranth is ranked as one of top five vegetables in antioxidant capacities (Walter et al., 2001). It contains plenty of bioactive components, such as *L*-ascorbic acid, beta-carotene, polyphenol, anthocyanins, lutein (Walter et al., 2001). It has been used as an antipyretic to reduce labor pain in Indian and Nepalese traditional medicine (Kirtikar et al., 1987). As astringent, diuretic, haemorrhage and hepatoprotective agent, amaranth has also been used to treat bladder distress, piles, tooth-ache, blood disorders and dysentery (Madhav et al., 2008). Amaranth has been evaluated for *in vivo* anthelmintic, anti-inflammatory and antioxidant properties (Lakshmanb et al., 2012).

Most vegetables including amaranth go through cooking such as frying, simmering, boiling, steaming, and blanching before consumption. Cooking has a significant effect on chemical compositions such as bioactive components, antioxidant activities and physical

characteristics in terms of color, texture and flavor. However, the effect can be either positive or negative depending on the process methods, vegetable species and shapes (Bernhardt et al., 2006). Amaranth is one of commonly consumed leafy vegetables in Guangdong Province, China. The vegetables are consumed after home cooking procedures such as simmering, frying, boiling, steaming, and blanching. Nevertheless, knowledge about its bioactive components, for instance, ascorbic acid, polyphenol, anthocyanins, carotenoids and antioxidant activities is limited. It is not clear how home cooking affects these bioactive components and their antioxidant capacities. Therefore, the current study aims to investigate

the contents of polyphenol, anthocyanins, ascorbic acid, carotenoids and antioxidant activities in terms of FRAP as affected by home cooking manners including simmering, frying, boiling, steaming and blanching.

2. Material and Method

2.1. Edible Amaranth

Edible amaranth was cultivated in a local farm in Zhuhai, Guangdong Province, China. A picture of the vegetable was shown in Figure 1.



Figure 1. Picture of leafy vegetable edible amaranth

2.2. Chemicals and Reagents

Acetate buffer, sodium hydroxide, citric acid, potassium chloride, dimethyl sulfoxide (DMSO), methylbenzene, hexane, acetone, methanol, heptane, isopropanol and ethanol were purchased from Tianjin Damao Chemical Reagent Co., Ltd (Tianjin, China). Gallic acid and ferric chloride were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Metaphosphoric acid and *L*-ascorbic acid were obtained from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin). Ferrous sulfate heptahydrate was provided by Luoyang Chemical Reagent Co., Ltd (Luoyang, China). 2,4,6-Tri[2-pyridyl-s-triazine] (TPTZ) were offered by Sigma- Aldrich Co. Ltd (St. Louis, MO, U.S.A). Folin-Ciocalteu reagent was from Shanghai Sanjie Biotechnology Co., Ltd (Shanghai, China).

2.3. Home Cooking of Edible Amaranth

The fresh raw amaranth was cleaned with tap water and drained with the paper towel. Three parallel portions of vegetable were sampled, and then subjected to each thermal processing in triplicate. The cooking time was selected based on the time used to obtain palatable vegetable. Briefly, for boiling treatment, 100 g cleaned amaranth was immersed in 1000 mL of boiling water for 3 min. For steaming treatment, 100 g cleaned amaranth was put on a steaming rack over 1000 mL water in a steamer covered with a lid, and steaming was conducted for 10 min. For simmering processing, 100 g cleaned amaranth was soaked into 1000 mL of boiling water, and then the vegetable was simmered at temperature about 94°C for 30 min over a slow fire. For pan-frying treatment, 100 g cleaned amaranth was added into the boiling corn oil at temperature around 232°C for 4 min. For blanching, 100 g edible amaranth was immersed with 1000 mL boiling water for 1 min. After cooking, water or oil was drained off from the samples. Cooked and fresh samples were homogenized by a food blender. All the crushed samples

were freeze-dried. The dry samples were stored at -20°C in a freezer for further analysis.

2.4. Determination of Total Phenolic Content (TPC)

The total phenolics were extracted and determined by a Folin-Ciocalteu assay using gallic acid (GA) as the standard by referring a previous publication (Xu and Chang, 2007). The absorbance was measured using a UV-visible spectrophotometer (TI-1901, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) at 765 nm against a reagent blank. The TPC was expressed as milligrams of gallic acid equivalents per gram of freeze-dried amaranth (mg of GAE/g).

2.5. Determination of Monomeric Anthocyanins Content (MAC)

The MAC was determined using a pH differential method (Liu et al., 2008) by measuring absorbance at 700 nm and 520 nm. The pigment content was expressed as micrograms of cyanidin-3-glucoside equivalents per gram of freeze-dried amaranth, using an extinction coefficient of $26900\text{ L cm}^{-1}\text{mol}^{-1}$ and molecular weight of 449.2 g mol^{-1} .

2.6. Determination of L-Ascorbic Acid

L-Ascorbic acid was extracted with meta-phosphoric acid according to the latest publication (Huang et al., 2014). Briefly, 0.5 g of freeze-dried raw or processed amaranth was ground with 13 mL of 3% meta-phosphoric acid in a blender and filtered through filter paper. An aliquot of filtrate containing 0.5 mg of ascorbic acid was sampled, and mixed with citrate-phosphate buffer (pH 3.6) to form buffered extract. The buffered extract was quickly mixed with indophenols solution and read absorption at 520 nm with 3% meta-phosphoric acid as blank. A calibration standard curve of ascorbic acid was used to calculate the total ascorbic acid with a unit of milligrams of ascorbic acid per gram of freeze-dried amaranth (mg/g).

2.7. Determination of Total Carotenoids

Total carotenoids content was determined according to a previous publication (Song and Xu, 2013). Briefly, the freeze-dried raw or processed amaranth (0.2 g) was extracted with 5 mL of acetone in a water bath at 50°C for 15 min. The mixture was centrifuged at 4200 rpm for 3 min. The supernatant was collected in 25 mL volumetric flask. The residue was extracted two more times with acetone under the same procedures. All the supernatants were collected in the 25 mL volumetric flask and bring to scale with acetone. 1 mL of extract was diluted by 9 mL acetone, and the total carotenoids were determined at 475 nm against the acetone blank.

2.8. Determination of Lutein

The lutein was determined using the method in the AOAC 43.018-43.023. Briefly, 0.2 g of freeze-dried edible amaranth was added into a 100 mL of volumetric flask with 1 mL distilled water and 30 mL of hexane, acetone, alcohol, methylbenzene (10:7:6:7). The sample was saponified with methanol and dilution with hexane.

Lutein and the phenyl (azo-1)-2-hydroxy beta (Sudan I) working solution as a calibration factor was measured at 474 nm.

2.9. Determination of Beta-Carotene

Beta-Carotene was determined according to Spirulina Pacifica Technical Bulletin #003b of Cyanotech Corporation. Briefly, 0.2 g freeze-dried edible amaranth was extracted in a tube with 2.5 mL DMSO by vortex. The solution was extracted with methanol for four times by centrifugation and then diluted into 25 mL solution with methanol. Saponification was conducted with 8 mL of the methanol extract, 5 mL of heptane and 1.5 mL of saturated KOH solution in the dark. Heptane was used twice to extract the carotenoids from above mixture. The beta-carotene was determined at 436 nm against a heptane blank.

2.10. Determination of Ferric-Reducing Antioxidant Power (FRAP)

The FRAP assay was performed as the method described previously (Xu and Chang, 2007). The FRAP value was expressed as microgram of Fe^{2+} equivalent per g of freeze-dried amaranth ($\mu\text{g/g}$) using the calibration curve of Fe^{2+} .

2.11. Statistical Analysis

The results were presented as the mean \pm standard deviation of three parallel measurements, and all statistical comparisons were made by means of ANOVA test and using SPSS software version 18 for windows (free version). It was considered as significantly different when $p < 0.05$ determined by Duncan's Multiple Range Test.

3. Result and Discussion

3.1. Effects of Home Cooking Manners on Polyphenol of Edible Amaranth

Total phenolic content in the edible amaranth was shown in Table 1. Different home cooking had extremely significant ($p < 0.001$) effect on polyphenol content on a dry weight basis. The decreasing sequence of polyphenol was steamed $>$ raw $>$ blanched $>$ boiled $>$ simmered $>$ fried. Steaming process increased the polyphenol content about 50%. The blanching, boiling and simmering method lowered the polyphenol content in the cooked amaranth, about 2.9%, 26.5%, and 31.1%, respectively. The frying caused a slight improvement (0.1%) in the polyphenol content in edible amaranth. Adithya (2012) proved that the total phenolic content and antioxidant activity of all the extracts of raw amaranth was higher than that of its blanched counterparts. About 82% of phenolic compounds were lost into the cooking water, after the green vegetables had been blanched for 15 min; the polyphenols had been broken down during cooking (Price et al., 1997). Therefore, the initial content of polyphenol was reduced by blanching, boiling and simmering. According to Chu et al. (2002), especially bound phenolics in total phenolics ranged from 20.5% in broccoli to 32.9% in cabbage. Bajorun (2004) reported that phenolics in vegetables also incurred in both free and conjugated forms. Generally,

fresh vegetables contain only conjugated flavonoids, and heat treatment increased the level of free flavonols

(Stewart et al., 2000). This can explain why frying and steaming increased the amount of the polyphenol.

Table 1. Total polyphenol, anthocyanins, L-ascorbic acid, and FRAP of raw and processed edible amaranth

Variables	Raw	Boiling	Steaming	Simmering	Frying	Blanching
Total polyphenol (mg/g)	1.94±0.04 b	1.43 ±0.04 d	2.96±0.02a	1.34±0.03e	1.94±0.03b	1.89±0.01c
Anthocyanins (µg/g)	50.9±0.82a	44.8±0.24c	46.1±0.48b	33.1±0.48d	6.15±0.08f	26.4e±0.55e
L-ascorbic acid (mg/g)	1.23±0.00c	1.29±0.00b	1.49±0.01a	1.00±0.01f	1.02±0.01e	1.06±0.00d
FRAP (µg/g)	302.5±4.2e	440.1±8.2d	1024.6±4.6a	445.7±9.5cd	603.4±10.0b	454.0±5.5c

3.2. Effects of Home Cooking Manners on Anthocyanins of Edible Amaranth

Anthocyanins content in edible amaranth was shown in Table 1. Cooking had an extremely significant effect on total polyphenol ($p < 0.001$). After cooking procedures, the content of anthocyanins was decreased, especially by frying. It obviously that the anthocyanins content in the edible amaranth ranked in raw > steamed > boiled > simmered > blanched > fried. 87.9% of its initial content was lost during frying in amaranth. Simmering determined a severe decrease of their initial concentration about 35.2% in amaranth, while the steaming and blanching caused a slight significant decrease 9.5% in anthocyanins content. Boiling reduced 12.1% of anthocyanins content in edible amaranth. According to Ahmed et al., (2010), 1.78 fold of anthocyanins of sweet potato declined after frying process, the sequence of anthocyanins was steamed > boiled > fried, which exhibited an identical trend as a result of edible amaranth by above cooking manners. Ninfali et al. (2005) reported that red onion was considered as the source of the anthocyanins and the severity of the cooking treatment for red onion was frying > boiling, because frying created a high temperature to destroy the anthocyanins. Temperature increase will see greater degree destruction in anthocyanins due to the hydrolyzation of the free glycoside structure (Laleh, 2006). Maccarone et al. (1985) studied the stability of anthocyanins in red orange juice in 15, 25 and 35 degree C during 15 day period storage and found that increase in temperature accelerated the destruction of anthocyanins.

3.3. Effects of Home Cooking Manners on Vitamin C of Edible Amaranth

Table 1 indicated the content of L-ascorbic acid in cooked amaranth. The raw edible amaranth contained vitamin C 1.23 mg/g on dry weight basis. Cooking had a significant ($p < 0.01$) effect on vitamin C. The reduction in vitamin C content could be attributed to the fact that it was water soluble and thermal instability (Oboh et al., 2005). The L-ascorbic acid ranged from 1.00 mg/g to 1.49 mg/g in amaranth. The descending order of the L-ascorbic acid in edible amaranth was steamed > boiled > raw > fried > simmered. Simmering, frying and blanching possessed a negative effect on the content of L-ascorbic acid (-18.6%, -17.2%, -14.0%) in amaranth. Attributed to the fact that in the initial time of boiling during 5-10 min, the maximum amount of vitamin C was destroyed or leached into the water; hence there was a small amount left in the vegetables during the later boiling time (Agbemafla, 2012). The boiling time of simmering was longer than that of blanching and boiling, as a result of the

most significant reduction of vitamin C by simmering. Frying had a negative effect (17.2%) on vitamin C content because the high temperature could destroy vitamin C. The steaming increased the content of vitamin C because of less vitamin C release from the cell tissue of amaranth. Yadav et al. (1995) reported that ascorbic acid content of fresh leaves was 6.24 to 6.29 mg/g amaranth (*Amaranthus tricolor*). A marked reduction in L-ascorbic acid and β -carotene was observed in dried, blanched and cooked leaves.

3.4. Effects of Home Cooking Manners on Carotenoids of Amaranth

The total carotenoid contents of raw and processed amaranth were presented in Figure 2. The total carotenoids content in raw amaranth was 1.42 mg/g. After cooking, total carotenoids content in amaranth was presented in descending order: blanched > simmered > boiled > steamed > fried > raw amaranth (shown in Figure 2). There was a significant ($p < 0.01$) effect of cooking on the content of total carotenoids in the edible amaranth, thus heating significantly increased the content of carotenoids. The total carotenoids contents in blanched, boiled and simmered amaranth were increased by 142.3%, 111.4%, and 111.3%, respectively. Khachik et al. (2008) found that boiling and frying manners promoted the content of carotenoids compared to the raw one. The reason was that the hydrophilic compound lost in the water whereas the hydrophobic compounds were released from the tissue of the tomato. For frying, the heating facilitated the carotenoids dissolved in the oil resulting in increasing the carotenoids content. Cooking of green fresh vegetables had been reported to promote the release of carotenoids from the matrix because of the disruption of carotenoid-protein complexes, leading to better extractability and high concentrations in cooked samples. The contents of all carotenoid compounds significantly increased in boiled and steamed broccoli (32% and 19%, respectively) in comparison to the raw ones. Conversely, frying determined a 67% loss of the initial carotenoid concentration, probably because of leaching of lipophilic compounds into oil and degradation by higher processing temperature (Miglio et al., 2008).

Contents of lutein in raw and processed amaranth were presented in Figure 2. The lutein content in raw amaranth was 1.25 mg/g. The lutein content in amaranth presented in a descending order: blanched > simmered > steamed > boiled > raw > fried. Blanching and simmering promoted the content of lutein. The blanching, simmering, steaming and boiling had a positive effect on the concentration of lutein, but frying reduced lutein content by 52.1%. The results are identical with the literature in which lutein content was increased from 168%-419% for red spinach

(*Amaranth gangeticus*) by thermal processing. This showed a good stability of lutein during boiling (Aman, 2005). Hart and Scott (1995) demonstrated that boiled spinach comprise of higher lutein content than the raw

ones. Miglio et al. (2008) reported that the lutein was lost by frying, and it has the same tendency as the current study. As a lipophilic compound, lutein was dissolved in heating oil, and then lost from the vegetables.

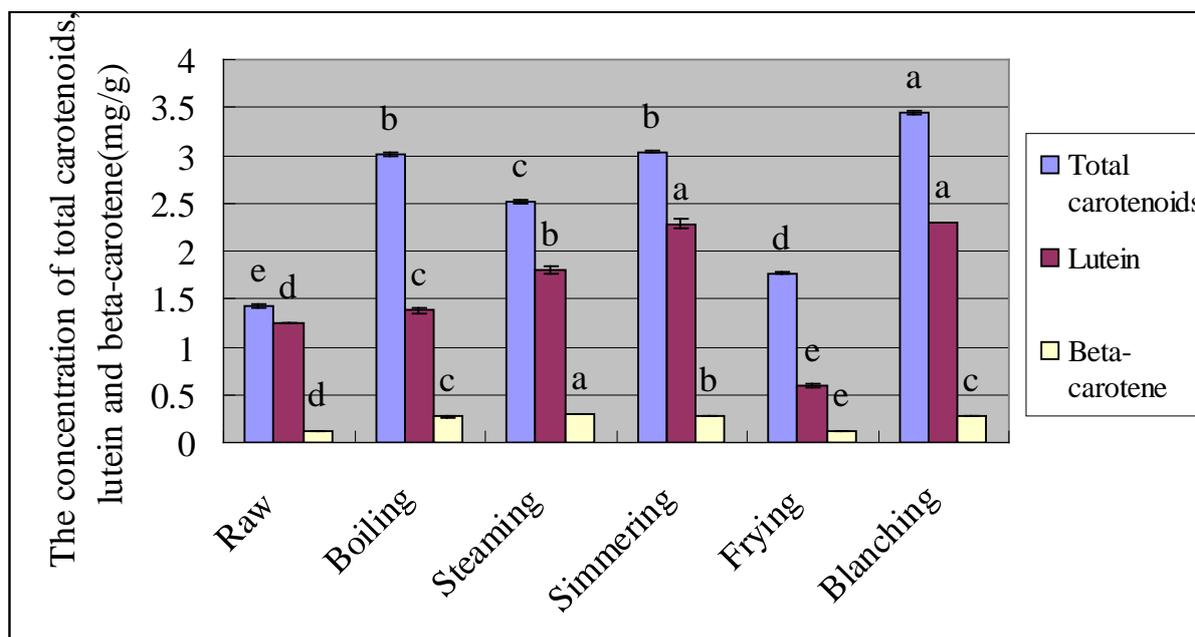


Figure 2. Total carotenoids, lutein and beta-carotene contents of raw and processed amaranth

The contents of the beta-carotene in raw and processed amaranth were shown in Figure 2. The beta-carotene in raw amaranth was 123.1 $\mu\text{g/g}$, the steamed amaranth presented the highest beta-carotene content (300.2 $\mu\text{g/g}$). Approximate one fold increases in the beta carotene for boiled, steamed, simmered, and blanched amaranth. There were extremely significant increase in the beta carotene by the thermal treatment, such as boiling, steaming, blanching, simmering ($p < 0.01$), but it had less significance effect by frying process ($0.01 < p < 0.05$). The steaming method had most positive effect on increasing the beta carotene content. Frying had a slight effect in decreasing the β -carotene (-9.4%) and moderate effect on lutein (-52.1%), but increased total carotenoids content. It was reported that both lutein and β -carotene were lost by frying. In conclusion, β -carotene had a higher thermal stability than lutein. Thus, more extractable β -carotene released through cell wall disruption in the case of suitable time and temperature (Miglio et al., 2008).

3.5. Effect of Home Cooking Manners on Antioxidant Activities of Edible Amaranth

The various FRAP values of raw and processed amaranth were shown in Table 1. The home cooking manners improved the FRAP values of amaranth from 302.5 $\mu\text{gFE/g}$ to 1024.6 $\mu\text{g FE/g}$. The decreasing order of the FRAP value was steamed > fried > blanched > simmered > boiled > raw amaranth. The FRAP values were affected mostly by steaming, while above one over three fold increases of the initial value of FRAP were found in boiled, simmered and blanched amaranth. The improvement of antioxidant activities was found in the processed amaranth because the antioxidant components, such as total polyphenol, *L*-ascorbic acid, total carotenoids, lutein and beta-carotene were all increased except for

anthocyanins. Gundgaard et al. (2003) concluded that boiling improved the FRAP level of all the vegetables as compared to the raw vegetable extracts. It was reported that total antioxidant activities of pepper, green beans, broccoli and spinach significantly ($p < 0.05$) were increased during cooking as compared to the values of the fresh ones (Gazzani et al., 1998). The steaming increased most of the nutrition such as vitamin C, beta-carotene, polyphenol, on the contrary the simmering destroyed most of the bioactive components.

4. Conclusions

Edible amaranth vegetable was consumed after home cooking such as boiling, frying, simmering, blanching and steaming. The health beneficial antioxidant activities are related to their bioactive components. Cooking had no deleterious effect on total bioactive component except for the reduction of anthocyanins content, but home cooking increase the antioxidant activities and the contents of carotenoids, especially by steaming. Steaming had a positive effect on the polyphenol, *L*-ascorbic acid, which lost seriously after simmering procedure. Both simmering and blanching increased the beta carotene and lutein in the cooked amaranth.

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