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Influence of Common Cooking Methods on Polyphenolic Composition and Antioxidant Activity of *Amaranthus dubius*

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ABSTRACT

Red spinach (*Amaranthus dubius*) is a nutrient-dense leafy vegetable known for its high content of polyphenolic compounds and antioxidant activity. However, the stability and bioavailability of these bioactives may be influenced by thermal processing. This study evaluated the effects of three common cooking methods; stir-frying, blanching, and steaming on the total phenolic content (TPC), total flavonoid content (TFC), betacyanin levels, and antioxidant activity of red spinach. Antioxidant potential was assessed using the DPPH radical scavenging assay, while phenolic constituents were profiled via high-performance liquid chromatography (HPLC). Stir-frying significantly enhanced antioxidant activity, achieving the highest DPPH scavenging value (50.79%), alongside increased TPC (86.40 mg GAE/100 g) and TFC (88.21 mg QE/100 g) compared to other treatments. Steaming and stir frying had improved phenolic retention, particularly preserving rutin, which recorded the highest concentration (10.39 mg/l and 8.15 mg/l, respectively) compared to other treatments. In contrast, betacyanin levels remained statistically unaffected across treatments. Overall, stir-frying demonstrated superior efficacy in enhancing antioxidant capacity and polyphenolic content. These findings provide valuable insights for optimizing cooking methods to maximize the nutritional quality of red spinach.

INTRODUCTION

The *Amaranthus* genus comprises more than sixty species distributed throughout tropical and subtropical regions. *Amaranthus dubius*, commonly known as red spinach is cultivated for its tender leaves and high nutritional value in regions such as Asia, Africa, Australia, and the Pacific Islands [1]. Red spinach is considered a nutrient-dense "superfood," rich in polyphenolic compounds, flavonoids, phenolic acids, vitamins (A and C), and essential minerals including calcium and iron [2,3]. These bioactive constituents contribute to its strong antioxidant potential and are associated with several health-promoting effects such

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as reduction of oxidative stress and prevention of chronic diseases. Moreover, *Amaranthus* species have long been utilised in traditional medicine for their anti-inflammatory, anti-ulcer, antimicrobial, and wound-healing properties [4]

The nutritional and functional properties of vegetables are profoundly influenced by cooking practices. Heat treatments such as blanching, steaming, and frying can alter the bioavailability and stability of phytochemicals and antioxidants [5,6]. Blanching, a short-duration heat process, can enhance polyphenol extractability by breaking down plant cell walls, whereas excessive exposure to boiling water may result in leaching of heat-sensitive and water-soluble nutrients [5,7,8]. Steaming is generally more effective in retaining antioxidant compounds due to minimal water contact, while dry-heat methods such as frying may variably influence polyphenolic retention depending on temperature and duration [9,10].

Although the effects of cooking on the antioxidant properties of various vegetables have been extensively studied, limited information is available on how different cooking methods affect the nutritional quality of red spinach. Understanding these effects is crucial to identifying optimal cooking practices that preserve its bioactive components. Therefore, the present study investigates the influence of blanching, steaming, and frying on the polyphenolic content and antioxidant capacity of red spinach (*Amaranthus dubius*). The findings are expected to provide valuable insights for consumers, researchers, and the food industry on how to retain the health-promoting properties of this underutilised leafy vegetable through appropriate cooking methods.

Materials and Methods

Materials

Fresh red spinach (*Amaranthus dubius*) or Chinese spinach (harvested after 30 days of planting) and palm oil were purchased from a local supermarket (Jaya Grocer, Plaza Shah Alam, Malaysia). Analytical-grade reagents included methanol, Folin–Ciocalteu reagent, sodium carbonate, ethanol, gallic acid, sodium nitrite, aluminum chloride hexahydrate, sodium hydroxide, Trolox, DPPH (2,2-diphenyl-1-picrylhydrazyl), formic acid, and rutin.

Sample preparation

Red spinach leaves were thoroughly washed under running tap water to remove surface impurities, drained, and cut into uniform sections (3 cm x 3 cm). The samples were divided into four treatments: raw (control), blanched, steamed, and stir-fried. Blanching was carried out according to Miškec et al. [5] and Severini et al. [8]. Approximately 100 g of red spinach was immersed in boiling water (100°C) for 3 minutes at a 1:4 (vegetable-to-water ratio), followed by rapid cooling in ice water (0°C) for 1 minute to halt enzyme activity. Steaming was performed based on Suwanwong et al. [9]. 100 g of red spinach was placed in a steaming basket over 200 ml of boiling water (100°C) for 5 minutes. Stir-fried sample was prepared according to Miškec et al. [5] with slight modification. 100 g of red spinach was cooked for 5 minutes using 200 g of palm oil at 160 °C, maintaining a 1:2 (vegetable-to-oil ratio) to prevent excessive oil absorption. All treated samples were cooled to room temperature, homogenized, and stored at −18 °C prior analysis.

Preparation of sample extract

The extract of all treated samples was prepared as followed the procedure of Suwanwong et al. [9] with modifications. A 5 g of homogenized sample was mixed with 50 ml of 80% methanol and vortexed for 15 minutes. The mixture was then centrifuged at 3500 rpm for 20 minutes. The supernatant was collected, stored in amber bottles, and kept at −18 °C until analysis.

Determination of Total Phenolic Content (TPC)

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Total phenolic content of samples was determined using the Folin–Ciocalteu colorimetric method [11]. An aliquot of 0.5 ml of extract was mixed with 2.25 ml of diluted Folin–Ciocalteu reagent (1:10 v/v) and allowed to stand for 5 minutes. Subsequently, 2.25 ml of 6% sodium carbonate was added and vortexed for 1 minute. After incubation in the dark for 30 minutes at room temperature, absorbance was measured at 725 nm using a UV–Vis spectrophotometer. The reagent blank contained 0.5 ml of 70% ethanol instead of the extract. Gallic acid standard of 20, 40 60, 80, and 100 mg/l was used to plot the standard curve graph. Results were expressed as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g fresh sample).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content of samples was quantified using the aluminium chloride colorimetric method [11]. A 0.5 ml aliquot of extract was mixed with 2.5 ml of distilled water and 0.15 ml of 5% sodium nitrite. After 6 minutes, 0.3 ml of 10% aluminium chloride solution was added and allowed to stand for 5 minutes. Then, 1 ml of 1 M sodium hydroxide was added, and the final volume was adjusted to 5 ml with distilled water. The solution was vortexed for 1 minute, and absorbance was measured at 510 nm. The blank contained 70% ethanol instead of the extract. Series of Trolox solutions (40, 60, 70, 90, and 100 µg/ml) were prepared. Results were expressed as milligrams of Trolox equivalents per 100 g of sample (mg TE/100 g FW).

Determination of Antioxidant Activity (DPPH Assay)

The radical scavenging activity of samples was determined using the DPPH method [9,12] A 0.1 mM DPPH solution was prepared in methanol. A 100 µl aliquot of extract was mixed with 3.9 ml of the DPPH solution and incubated in the dark for 30 minutes at room temperature. Absorbance was recorded at 517 nm. The percentage inhibition of DPPH radicals was calculated using the equation:

$$\% \text{ DPPH Inhibition} = A_{control} - A_{sample} / A_{control} \times 100$$

Where, $A_{control}$ is the absorbance of DPPH without extract and A_{sample} is the absorbance of DPPH with extract. All measurements were performed in triplicate.

Determination of rutin compound using HPLC-DAD

Rutin compound in all samples were analysed using High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) [13]. Separation was achieved using a C18 column (150 mm × 4.6 mm i.d., 5 µm). The mobile phase consisted of solvent A (0.2% formic acid in water) and solvent B (methanol), with a gradient elution as follows: 0 minute, 85% A:15% B; 5 minutes, 75% A:25% B; 20 minutes, 60% A:40% B; 30 minutes, 40% A:60% B; 35 minutes, 10% A:90% B; 37 minutes, 0% A:100% B; 39 minutes, 100% A:0% B; 40 minutes, re-equilibrated to 85% A:15% B. The flow rate was 1 ml/min, and detection was performed at 270 nm. The injection volume was 10 µl for both standard and sample solutions. Quantification was based on standard additional method with rutin standard of 0, 50, 200, and 250 mg/l was injected into sample at various treatment.

Determination of betacyanin content

Betacyanin concentration of all samples was determined calorimetrically as described by Constantin et al. [14] and Kumar et al. [15], with minor modifications. 100 g of homogenized sample was mixed with 50 ml of 80% methanol and vortexed for 20 minutes. The mixture was centrifuged at 3500 rpm, and the supernatant was collected. Absorbance was measured at 538 nm using a UV–Vis spectrophotometer, with 80% methanol used as the blank. Betacyanin content was calculated using the formula:

$$\text{Betacyanin (mg/L)} = A \times MW \times DF \times 1000\varepsilon l$$

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Where, A = absorbance at 538 nm, MW = molecular weight of betanin (550 g/mol), DF = dilution factor, ε = molar extinction coefficient of betanin (65,000 L/mol·cm), and l = path length of cuvette (1 cm).

Statistical Analysis

All analyses were performed in triplicate, and data were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA in IBM SPSS Statistic Software 21. Post Hoc Tukey Test was used to determine differences among means at $p < 0.05$.

RESULTS AND DISCUSSION

Phenolic content and antioxidant activity

Cooking treatments significantly influenced the total phenolic content (TPC), total flavonoid (TFC), and DPPG scavenging activity of red spinach (Fig. 1). Stir-fried samples showed the highest TPC (86.40 mg GAE/100 g), followed by steamed (78.93 mg GAE/100 g) and raw (47.21 mg GAE/100 g), while blanching yielded the lowest value (31.47 mg GAE/100 g) as shown in Fig. 1a. The increase in TPC observed in steamed and stir-fried samples is attributed to the heat-induced disruption of cell walls, which enhances the release of bound phenolics and increases extractability. Steaming preserves phenolics by minimising direct water contact, thus reducing leaching losses [7]. In stir-frying, elevated temperature and oil facilitate the release and solubilization of lipophilic phenolics, which may enhance their bioavailability [16]. Conversely, blanching caused marked losses in TPC, likely due to leaching of water-soluble and thermolabile compounds into the cooking medium [5,6,17].

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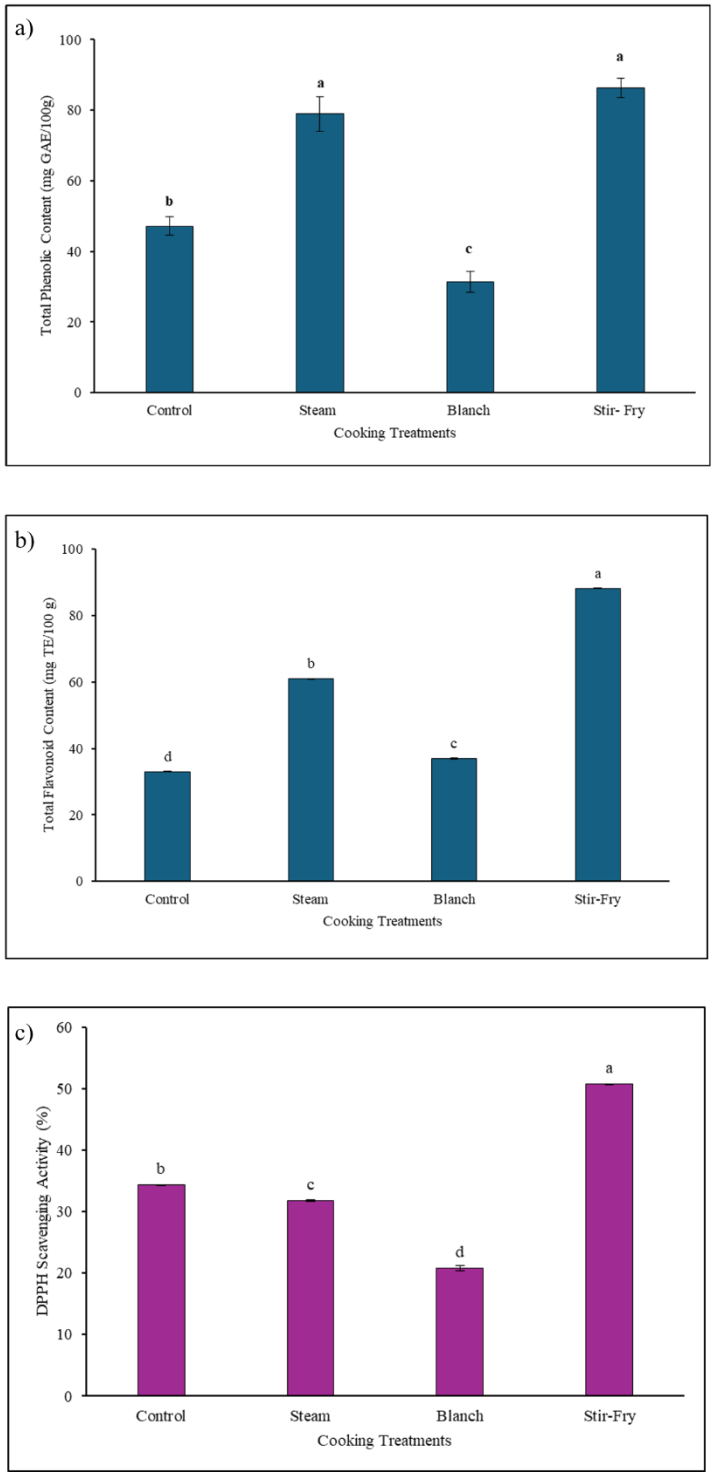

Fig. 1. Total phenolic content (a), total flavonoid content (b) and DPPH scavenging activity (c) of different cooking treatments of red spinach (*Amaranthus dubius*).

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The similar trend was observed in total flavonoid content as shown in Fig. 1b, stir-fried red spinach exhibited the highest total flavonoid content (88.21 mg QE/100 g), followed by steamed (61.03 mg QE/100 g), blanched (36.99 mg QE/100 g), and raw samples (33.03 mg QE/100 g). The substantial increase in TFC after stir-frying can be explained by thermal disruption of plant tissues, which enhances the liberation of flavonoids from the cellular matrix [8]. The presence of oil promotes the solubilisation of lipophilic flavonoids and may protect certain compounds from oxidation during cooking [16]. Steaming also retained a high level of flavonoids due to limited leaching and shorter heat exposure [9].

In contrast, blanching led to moderate losses because of the solubility of flavonoids in hot water and their degradation during exposure to moist heat [18]. These results align with previous findings that non-aqueous cooking methods such as stir-frying can increase flavonoid availability by improving extractability and transformation of complex glycosides into simpler, more bioactive forms [5]. Although stir-frying may increase oil uptake, it simultaneously enhances flavonoid release, suggesting a trade-off between nutritional gain and lipid incorporation.

Fig. 1c shows that Stir-fried samples exhibited the highest radical scavenging activity (50.79%) as compared to other treated samples. The enhancement in antioxidant activity of red spinach after stir-frying corresponds with the elevated levels of phenolic (Fig. 1a) and flavonoid (Fig. 1b) compounds, as both are major contributors to radical scavenging capacity [9]. Heat treatment during stir-frying likely disrupts cellular barriers, facilitating the release of bound antioxidants. Moreover, the oil matrix may dissolve lipophilic antioxidants, improving their recovery and potential bioavailability [16]. The reduced antioxidant activity observed in blanched (20.79%) and steamed (31.81%) samples as compared to control (34.30%) is attributed to the degradation and leaching of water-soluble antioxidants, including vitamin C and polyphenols [7]. Steaming, however, retained higher antioxidant capacity than blanching, confirming that non-immersive cooking helps minimize compound loss.

Collectively, stir-frying is the most efficient technique in preserving or even enhancing phenolic and flavonoid content as well as DPPH scavenging activity. This outcome underscores the importance of cooking method selection for retaining bioactive compounds linked to antioxidant, anti-inflammatory, and anti-carcinogenic effects [5,6,19].

Rutin content

Quantification of rutin revealed significant differences ($p<0.05$) among cooking treatments (Table 1). Steamed samples recorded the highest rutin concentration (10.39 mg/l), followed by stir-fried (8.15 mg/l) samples. The increase in rutin content after steaming and stir-frying treatments supports the hypothesis that mild heat enhances the release of bound phenolics and improves extractability of raw sample [20]. Steaming minimises leaching and oxidation, preserving heat-sensitive flavonoids [9], whereas the oil medium during stir-frying protects rutin from thermal degradation and promotes solubilisation. The absence of detectable rutin in blanched samples indicates substantial leaching or degradation in aqueous conditions, as rutin is water-soluble and thermolabile [7]. This trend aligns with the overall phenolics and antioxidant results (Fig. 1), confirming that mild or non-aqueous cooking methods such as steaming and stir-frying are optimal for preserving rutin and related flavonoids.

Table 1. Rutin content (mg/l) of different cooking treatments of red spinach (*Amaranthus dubius*).

Cooking treatment	Rutin Concentration (mg/l)
Control	3.91 ± 1.95^{b}

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Steam	10.39 ± 4.84[a]
Blanch	Not Detected
Stir-Fry	8.15 ± 6.01[ab]

Values of mean ± SD (n=3). Different superscript letters indicate significant differences within cooking treatments at $p < 0.05$.

Betacyanin content

Fig. 2 shows that the cooking treatments did not significantly ($p>0.05$) influence the betacyanin content in red spinach. The control sample was accounted 2.68 mg/ml, followed by steamed (2.36 mg/ml), blanched (1.97 mg/ml), and stir-fried (1.94 mg/ml) samples, respectively. The minor decline in betacyanin after cooking indicates that these pigments exhibit relative thermal stability under short-time heat treatments. It is supported by Prabhu et al. [21] as reported that betacyanin can tolerate mild heating when oxygen exposure is limited. Moreover, lack of significant loss during frying may be due to the reduced water activity in oil, which limits pigment leaching and oxidation. Although betacyanin are sensitive to temperature and pH [22], their stability in red spinach suggests a robust matrix protection effect. Preservation of betacyanin content is nutritionally relevant since these pigments contribute not only to the visual appeal of red spinach but also to its antioxidant and health-promoting properties.

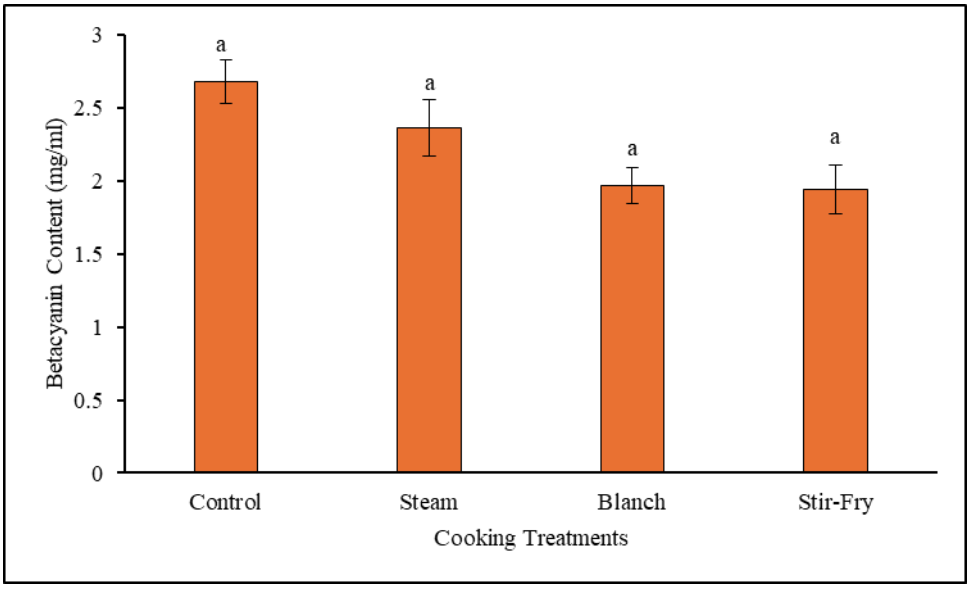

Fig. 2. Betacyanin content (mg/ml) of different cooking treatments of red spinach (*Amaranthus dubius*).

CONCLUSION

This study demonstrated that different cooking methods markedly influence the retention of bioactive compounds and antioxidant properties in red spinach (*Amaranthus dubius*). Stir-frying and steaming significantly enhanced total phenolic and flavonoid contents, as well as overall antioxidant activity. The increase is attributed to the improved release and extractability of bound phytochemicals through heat-induced cell wall disruption and the protective role of oil or steam in minimizing degradation. These findings emphasize that steaming and stir-frying are appropriate domestic cooking methods to preserving

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or enhancing the nutritional and functional value of red spinach, supporting its promotion as a health-promoting leafy vegetable.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders

AUTHORS' CONTRIBUTION

Nureen Aisyah Saipuddin conceptualised the central research idea, carried out the research, wrote and revised the article. Siti Rashima Romli provided the theoretical framework, designed the research, supervised research progress, anchored the review, revisions and approved the article submission.

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