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Influence of processing on tannin, flavonoid, polyphenol content, and antioxidant activity of Ivy gourd (*Coccinia grandis* (L.) Voigt) leaf tea

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ABSTRACT

This study investigates factors affecting tea production from the leaves of Ivy gourd (*Coccinia grandis* (L.) Voigt) using a natural fermentation method. To evaluate the tea quality, key parameters such as tannin, flavonoid, polyphenol content, and antioxidant activity were monitored throughout the research. The study examined different factors, including leaf moisture loss prior to fermentation, the fermentation temperature and duration, the roasting temperature, and the final product moisture content. The results indicated that drying the leaves to 80% moisture content facilitated the natural fermentation process at 40 °C for 5 h. Roasting the tea at 100 °C to a final moisture of 8% maintained a stable content of tannin, flavonoid, polyphenol, and oxidation capacity.

Keywords: Antioxidant activity, *Coccinia grandis* (L.) Voigt, polyphenols, tea.

INTRODUCTION

Ivy gourd or scarlet gourd (*Coccinia grandis* L.) is a plant species widely distributed in Vietnam and can be used in salads or cooked in curry [1, 2]. Ivy gourd features many valuable bioactive properties, including anti-inflammatory effects, antioxidant activity, and antimicrobial effects [3-5]. Previous studies demonstrate its ability to reduce blood glucose levels in rat models and regulate blood lipids [1, 6, 7].

Tea is the world's second most widely consumed beverage after water [8, 9]. Tea consumption is an ancient and traditional practice that originated in China and India about 5,000 years ago [10, 11], and tea is made from dried leaves of plants. Different types of tea are classified according to the degree of fermentation and have different characteristics, including color, appearance, flavor, and aroma [12].

Studies have shown that the polyphenol content in tea lowers the chance of developing some chronic illnesses, including cancer and heart disease [11, 13-14]. Tea also helps control body weight, promotes bone and oral health, prevents kidney stones, and provides protection against ultraviolet radiation among other physiological benefits [11, 14-16]. These health benefits are mainly due to the polyphenols present in tea, which remain after fermentation and retain their beneficial effects on health [17].

Tea processing typically includes withering, fermentation, preliminary drying, and roasting, in which fermentation plays an important role in determining tea quality. Tea fermentation is a series of reactions of leaf components under the influence of enzyme activity at certain temperature and moisture [18]. Under the impact of polyphenol oxidase and peroxidase, catechins are oxidized into quinones, which are then further oxidized and condensed into theaflavins, theasinensins, thearubigins, and theabrownins [19, 20]. This significantly impacts tea products' flavor, liquidity, appearance, color, and biological properties [21, 22]. Temperature is known to be one of the key factors of fermentation and is reported to directly affect the conversion of catechin and the formation of theaflavin, thearubigin, and theabrownin [23]. Currently, most studies related to the black tea fermentation process focus on the changes in external factors such as temperature [24, 25], moisture [26], time [27], and oxygen supply [28, 29] to control the extent and quality of the fermentation process. Studies on fermentation temperature mainly focused on its effects on flavoring substances [30] and aroma components [25]. Research on the factors of tea processing that affect tannins, flavonoids, polyphenols, and antioxidant capacity in Ivy gourd leaves has yet to be thoroughly studied. Therefore, this study aims to investigate the parameters affecting the content of tannins, flavonoids, polyphenols, and antioxidant capacity during the processing of Ivy gourd tea leaves.

EXPERIMENTAL

Plant material: The ivy gourd leaves were harvested in the morning from the experimental garden of the College of Agriculture, Can Tho University. The raw materials must be intact, free from mud and dirt, clean, and free from bruising, wilting, or pest damage. After harvesting, the leaves were washed, dried, and used for further experiments.

Extraction method: This study pertains to tea products, thus the extraction of biocompounds such as total polyphenol content (TPC), total flavonoid content (TFC), and tannins is modeled after the traditional tea brewing process. The extraction is conducted using water as the solvent. A 5 g of the sample after processed with different conditions below was weighed. 250 mL of distilled water was simultaneously prepared and heated to boiling point using a microwave (Sharp, model R20A1 S(VN), Thailand) for 2 minutes at 800 W. The boiling water was poured into the cup containing the tea, ensuring the water level was 4-6 mm below the rim of the container. The cup was covered and left to sit for 6 minutes. After extraction, the sample was removed and vacuum filtration was performed to eliminate fine residues. The obtained volume was recorded. The extract was then used to determine tannins, flavonoids, polyphenols, and antioxidant capacity.

Determination of the optimal leaf maturity for processing Ivy gourd tea: The leaves at different stages of maturity were collected, cleaned, dried, and separated into two parts:

Part 1: The leaves were finely ground to create fresh Ivy gourd leaf powder, and the initial moisture content was determined. Fresh leaf powder was extracted using the previously described method. The extract was used to determine the content of tannins, flavonoids, polyphenols, and antioxidant capacity.

Part 2: Leaves at different levels of maturity were cut into strips about 1 cm long. After that, the leaves were fermented at 40 °C for 4 hours, then roasted at 100 °C until the product moisture content was about 6%. The finished tea was stored in vacuum-sealed PA packaging and kept in a refrigerator (at a temperature lower than 10 °C) until further analysis.

Effect of moisture on the effectiveness of the natural fermentation process: Leaves obtained optimum ripening were cleaned and withered by hot air drying at 50 °C to moisture reduction levels of 5%, 10%, 15%, and 20%. Then, the Ivy gourd leaves were fermented at 40 °C for 4 h and roasted at 100 °C until the product moisture content was about 6%. The finished tea was then finely ground, and the levels of tannins, flavonoids, polyphenols, and antioxidant capacity were determined.

Effect of temperature and fermentation time on the quality of Ivy gourd leaf tea: Leaves obtained optimum moisture reduction levels were then fermented at 30 °C, 40 °C, and 50 °C for different durations (4, 5, and 6 h). Roasting and analysis were conducted as described above.

Effect of roasting conditions on the quality of Ivy gourd leaf tea: Leaves processed under optimal fermentation conditions were roasted at different temperatures (80 °C, 90 °C, and 100 °C) until the product's moisture content reached (6%, 8%, and 10%). The finished tea samples were evaluated for the content and properties of bioactive compounds.

Analysis method: Moisture content was determined as a percentage (g/100 g). The sample is dried in an oven at 103 °C to constant weight. The free water content is the mass lost after drying to constant weight. Total polyphenol content (TPC, mg GAE/g DW) was quantified using the colorimetric method with gallic acid as the standard, Folin-Ciocalteu reagent as the oxidizing agent, and absorbance measured at a wavelength of 738 nm [31].

Antioxidant activity (AC, µmol TE/g DW) was assessed using a free radical method with DPPH (2,2–Diphenyl-1-picrylhydrazyl) as the standard, and results were compared to Trolox. The color change from purple to light yellow was measured at a wavelength of 517 nm [32]. Total tannin content (TTC, mg GAE/g DW): Based on the reaction of tannin with Folin-Ciocalteu reagent, measuring the color absorption at 725 nm wavelength [33]. Total flavonoid content (TFC, mg QE/g DW): Spectrophotometric method at 415 nm wavelength, based on flavonoid-aluminum complex formation reaction [34].

Statistical data analysis: All the experimental results were analyzed using Statgraphics Centurion 16.2 (USA). All experiments were conducted in triplicate. Analysis of variance (ANOVA) with the least significant difference procedure was used to determine the significant differences ($p \leq 0.05$) between means.

RESULTS AND DISCUSSION

Effect of leaf maturity on the production of Ivy gourd leaf tea: The quality of tea products depends not only on the processing stages but also on the raw materials used. Numerous studies have shown that, in addition to factors such as cultivars, cultivation practices, climate, and soil conditions, the maturity of the raw material has a significant impact on their properties [35]. Ivy gourd were collected and tested at three different maturity stages, specifically young, developing, and mature leaves. The criteria for distinguishing these three types of leaves are presented in Table 1.

The experimental results in Fig. 1 show a significant difference in polyphenol content in the initial raw materials. During the leaf formation stage, biochemical reactions occurring within plant cells lead to changes in the formation and synthesis of chemical components at different stages of leaf development [36]. Young leaves had higher polyphenol content and antioxidant activity than developing and mature leaves.

Table 1. Differentiation of Ivy gourd leaves at different maturity stages

Features	Young leaves	Developing leaves	Mature leaves
Identification characteristics	The leaves are green with a yellowish hue, the undersurface is hairy, the outer edges are serrated, and the leaves and stalks are soft.	The entire leaf is green, the undersurface is hairless, the leaf edge is smooth, the leaf is thin, and the leaf stalk is tough.	The entire leaf is dark green, slightly tinged with black, hairless, smooth leaf edges, crispy, thick, very tough leaf stalk.
Length (cm)	2.44 ± 0.48	4.13 ± 0.56	7.27 ± 0.69
Width (cm)	1.71 ± 0.32	3.18 ± 0.38	5.38 ± 0.62
Number of leaves/10 g	30 - 50	12 - 20	7 - 10
Photos			

According to Zagoskina *et al*., polyphenols are products of the photosynthesis process and are closely related to light intensity [37]. As light intensity increases, polyphenol content in the raw materials also increases. Young leaves are located on the top, often crawling on the older leaf surface and reaching out to places with light to perform photosynthesis. They also climb other trees to reach sunlight. Therefore, these young leaves receive the best light conditions and often have higher polyphenol content than older leaves at the base. Similarly, the experimental results presented in Fig. 2 show that the TFC and TTC of fresh Ivy gourd leaves had statistically significant differences at various leaf aging stages. Young leaves had the highest total flavonoid and tannin content compared to the two older leaf levels. This suggests that the biological activity of scarlet gourd leaves changes depending on each stage of leaf maturity. Young leaves are vulnerable to environmental stressors, so the natural defense mechanism of the plants produces higher levels of polyphenolic compounds to protect the leaves.

Effect of the moisture content of Ivy gourd leaves on tea fermentation: Withering or reducing the moisture content of the leaves prior to fermentation is a crucial step. Reducing the moisture content to an optimal level in tea processing facilitates easier control during fermentation, which is beneficial for the biochemical changes involved in black tea production [38].

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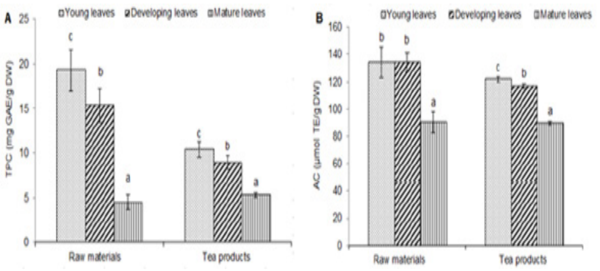

Fig. 1. Changes in polyphenol content (A) and antioxidant activity (B) of raw materials at different maturity levels and the final tea product.

Note: Different letters in the same column indicate significant differences between the samples at the 95% confidence level.

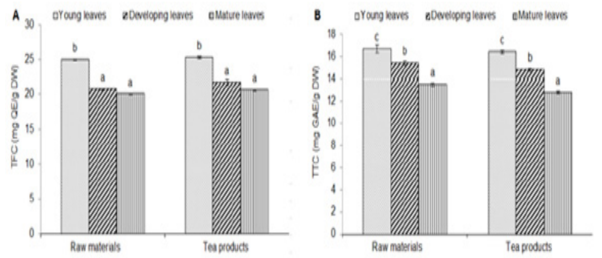

Fig. 2. Changes in total flavonoid (A) and total tannin content (B) of Ivy gourd leaves at different maturity levels of raw materials and the tea product.

Note: Different letters in the same column indicate significant differences between the samples at the 95% confidence level.

changes involved in black tea production [38].

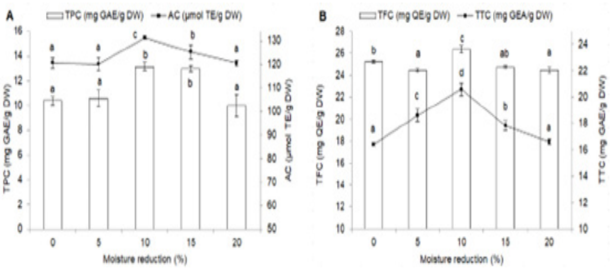

Fig. 3. Effect of moisture reduction on TPC-AC (A), TFC-TTC (B) of Ivy gourd leaves.
Note: Different letters in the same column indicate significant differences between the experimental treatments at the 95% confidence level.

Moisture reduction significantly affects the moiture, TPC, AC, TFC, and TTC in Ivy gourd tea leaves (Fig. 3). A 10% and 15% moisture reduction resulted in the highest polyphenol content. At moisture reductions of 0% and 5%, the roasting process of the tea was prolonged, creating unfavorable conditions for chemical transformations. The AC at 10% moisture loss was higher than that at 15% moisture loss. The extended withering process at 15% moisture reduction decreased the antioxidant activity of the product due to the degradation of these compounds by oxygen in the air. Similarly, the highest levels of flavonoids and tannins were achieved when the moisture reduction was at 10%. Moisture reduction to 20% decreased the efficiency of extracting bioactive compounds. Moisture reduction to 20% decreased the efficiency of extracting bioactive compounds, likely due to changes in polyphenol oxidase (PPO) activity, which influenced TPC levels. This trend is consistent with the findings of Turkmen et al. that factors in tea processing affecting polyphenol content are closely linked to enzymatic activity in materials [39].

It made the tea leaves more brittle during the rolling process, a crucial step for releasing the enzymes and substrates necessary for fermentation. The products generated during natural fermentation, including compounds belonging to the tannin and flavonoid groups, play an important role in flavor development during subsequent processing [38].

Based on the results, a moisture reduction of 10% (with a moisture content of approximately 80-81%) can be considered suitable for the natural fermentation process in Ivy gourd tea production.

Effect of temperature and time on the efficiency of natural fermentation: According to *Zhu et al*., temperature plays a vital role in controlling the rate and extent of chemical reactions occurring during fermentation [40]. Temperature directly impacts enzyme activity, influencing the oxidation of compounds crucial for tea's color and flavor. Proper temperature control ensures desired tea characteristics. Fermentation leads to substantial changes in the chemical composition of green tea leaves. This is an important step in the tea production process. This is an important step in the tea production process. Temperature variations can influence the antioxidant activity of plant-based foods, such as by altering the production of phenolic compounds [41].

Furthermore, the conversion of catechins or flavanols into simpler compounds such as theaflavins and thearubigins, play an important role in the development of color and flavor in the finished tea product [42]. Creating favorable conditions for fermentation is key to obtaining a product with good sensory qualities.

The experimental results on the changes in TPC, AC, TFC, and TTC of Ivy gourd leaves with varying fermentation times and temperatures are presented in Table 2. The results show that at a temperature of 40 °C for 5 h, the highest TPC was achieved, and the AC was also better compared to samples at other temperatures

Table 2. Changes in TPC, AC, TFC, and TTC at different fermentation times and temperatures

Temp. (ºC)	Time (hrs)	TPC (mg GAE/g DW)	AC (µmol TE/g DW)	TFC (mg QE/g DW)	TTC (mg GEA/ g DW)
30	4	13.14^{b} ± 0.36	128.86^{abc} ± 1.12	26.04^{b} ± 0.12	18.61^{ab} ± 0.28
	5	13.01^{b} ± 0.44	129.52^{bc} ± 0.98	26.45^{cd} ± 0.24	18.45^{ab} ± 0.35
	6	12.24^{a} ± 0.38	127.5^{a} ± 0.87	26.47^{de} ± 0.16	18.58^{ab} ± 0.34
40	4	13.17^{b} ± 0.35	131.33^{d} ± 0.81	26.35^{e} ± 0.34	20.60^{b} ± 0.58
	5	14.06^{c} ± 0.24	134.44^{e} ± 0.96	28.84^{f} ± 0.49	20.57^{c} ± 0.64
	6	13.40^{bc} ± 0.36	128.68^{ab} ± 1.01	26.53^{d} ± 0.35	19.20^{b} ± 0.53
50	4	13.66^{bc} ± 0.34	131.24^{d} ± 1.06	26.42^{cd} ± 0.18	18.74^{ab} ± 0.29
	5	13.56^{bc} ± 0.50	130.46^{cd} ± 1.03	26.18^{bc} ± 0.26	17.63^{ab} ± 0.45
	6	13.26^{b} ± 0.45	129.75^{bcd} ± 0.53	25.49^{a} ± 0.44	17.92^{ab} ± 0.66

Note: Different letters in the same column indicate significant differences between the experimental treatments at the 95% confidence level.

and times. Extended fermentation at 50 °C could reduce both the TPC and AC of polyphenolic compounds.

The effectiveness of the fermentation process heavily relies on reactions catalyzed by intracellular enzymes [43]. Both excessively high and low reaction temperatures are unfavorable for enzyme-mediated reactions. A similar effect can be anticipated with fermentation time, where both too short and too long fermentation times are detrimental. Short fermentation times do not allow sufficient time for reactions to occur fully, while extended fermentation times lead to the degradation of polyphenolic compounds due to their reaction with oxygen in the air.

The analysis results in Table 2 show that, when considering different compounds over time, a temperature of 40 °C for 5 h yields the optimal results for achieving the highest TPC, AC, TFC, and TTC in the final tea product.

Effect of moisture content and temperature during the roasting process on the TPC, AC, TFC, and TTC of Ivy gourd tea leaves: In agricultural countries, large quantities of products and foods are roasted or dried to enhance storage capacity, preserve their original value, maintain nutritional content, and reduce packaging costs [44]. For instance, high-temperature roasting and fermentation can enhance the nutritional profile and phytochemical content of Glycine max (Soybean) seed, potentially conferring health benefits [45]. The roasting process involves the removal of water using hot air with relatively low humidity. Roasting plays an important role in tea production and storage. Roasting at high temperatures deactivates enzymes, stopping the biochemical reactions in the natural fermentation of tea leaves while promoting reactions that produce color and flavor (such as the Maillard reaction and caramelization). These reactions help the products achieve their characteristic sensory value. The compounds formed during these reaction chains are responsible for changes in TPC and AC in the tea. Roasting also removes water from the product, reducing its water activity, which in turn halts biochemical processes and microbial growth.

The results presented in Table 3 indicate that at different roasting temperatures and moisture content levels, the samples' polyphenol content and biological activity were different. At 90 °C roasting temperature, polyphenol content loss was consistently higher than at other temperature modes. At roasting temperatures of 100 °C and 110 °C, excessively low and excessively high moisture content resulted in a loss of polyphenols. This finding is consistent with previous studies [46], where similar changes were observed under comparable conditions. This can be explained by the fact that under low-temperature conditions or when roasting is stopped at low moisture, the roasting time is longer, increasing the exposure time of the products to air oxygen, thereby causing the loss of polyphenols. Conversely, if roasting is stopped at higher moisture levels, the higher water content in the tea can affect the storage conditions prior to analysis.

Table 3. Changes in TPC, AC, TFC, and TTC at different fermentation times and temperatures

Temperature (°C)	Moisture (%)	TPC (mg GAE/g DW)	AC (µmol TE/g DW)	TFC (mg QE/g DW)	TTC (mg GEA/ g DW)
90	6	$13.56^{a} \pm 0.46$	$137.59^{b} \pm 1.50$	$27.34^{d} \pm 0.10$	$18.07^{a} \pm 0.13$
	8	$14.33^{cd} \pm 0.25$	$136.54^{ab} \pm 1.67$	$27.59^{e} \pm 0.13$	$18.25^{b} \pm 0.14$
	10	$13.73^{ab} \pm 0.30$	$137.46^{b} \pm 2.30$	$27.30^{cd} \pm 0.15$	$18.47^{c} \pm 0.31$
100	6	$14.06^{bc} \pm 0.28$	$134.44^{a} \pm 2.27$	$28.84^{f} \pm 0.34$	$20.57^{f} \pm 0.64$
	8	$14.98^{e} \pm 0.24$	$143.54^{d} \pm 1.48$	$29.64^{g} \pm 0.28$	$21.41^{g} \pm 0.46$
	10	$13.40^{a} \pm 0.26$	$139.23^{bc} \pm 1.40$	$27.06^{cd} \pm 0.15$	$19.35^{e} \pm 0.35$
110	6	$14.29^{cd} \pm 0.22$	$134.14^{a} \pm 2.09$	$27.16^{b} \pm 0.09$	$19.02^{d} \pm 0.19$
	8	$15.01^{e} \pm 0.25$	$142.07^{cd} \pm 1.21$	$27.22^{c} \pm 0.29$	$19.44^{e} \pm 0.23$
	10	$14.71^{de} \pm 0.27$	$138.11^{b} \pm 1.11$	$26.95^{a} \pm 0.16$	$18.29^{b} \pm 0.22$

Note: Different letters in the same column indicate significant differences between the experimental treatments at the 95% confidence level.

In summary, roasting at 100 °C with moisture content at 8% and roasting temperature at 110 °C with moisture content at 8% had higher polyphenol content and biological activity than at other temperature and moisture levels. However, roasting mode at 100 °C and moisture content controlled at 8% gives higher AC and should be chosen in the tea processing process from Ivy gourd leaves. Likewise, as Juan [47] reported, Oolong tea roasted at 140 °C or higher exhibited significant quality degradation, manifested in a red liquor, sour taste, and burnt aroma. Juan proposed that a temperature range of 100-120 °C is optimal for a mid-grade tea.

In addition, Table 3 also shows that the TFC and TTC reached the highest value in the sample roasted at 100 °C until 8% moisture content. Roasting at 90 °C was insufficient to develop the tea product's quality, resulting in lower flavonoid and tannin recovery. At the same time, the 110 °C temperature promoted the oxidation process to occur more strongly and was more challenging to control. The 6% moisture level also prolonged the roasting time and did not have the optimal flavonoid and tannin content.

Based on the research results, it can be concluded that the roasting mode at 100 °C until reaching 8% moisture content gives finished tea with the highest tannin and flavonoid content, as well as the highest polyphenol content and antioxidant activity.

In general, the technological process of producing Ivy gourd tea with optimal parameters is summarized as follows: Fresh young leaves → Withering (50 °C, moisture ~ 80%) → Short fermentation (40 °C, 5 h) → Roasting (100 °C, moisture ~ 8%) → Ivy gourd tea.

CONCLUSIONS

The research results showed that each stage in the processing of Ivy gourd leaves tea significantly affects the TPC, AC, TFC, and TTC. Using young Ivy gourd leaves as raw material for making tea, combined with the moisture reduction at the stage of withering by 10% before fermentation, followed by fermentation at 40 °C for 5 h gives the highest efficiency in obtaining TPC, AC, TFC, and TTC. Additionally, roasting the scarlet gourd leaves at 100 °C to a moisture content of 8% yields a high-quality tea product with TPC of 14.98 mg GAE/g DW and AC of 143.54 μmol TE/mg DW; TFC and TTC reach 29.64 mg QE/g DW and 21.41 mg GEA/g DW, respectively. This study establishes a scientific basis for producing high-quality herbal tea, demonstrating its potential as a functional beverage with significant nutritional and antioxidant properties.

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