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# Influence of pretreatment methods and addition of fragrant plants on *Gnetum gnemon* var *tenerum* powder tea

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**ABSTRACTS**

**Background:** Enhancing the utility of fresh vegetables, such as Liang leaves, is commonly achieved through drying and powdering as functional ingredients and food. In a preliminary study, Liang leaves displayed similar color, appearance, and texture attributes to Japanese and Chinese teas, but the flavor of the Liang drink significantly diminished. This research highlights the potential of the highly sought-after gastronomic vegetable, *Gnetum gnemon* var *tenerum,* as Liang, a Thai indigenous vegetable, but there is still a shortfall of scientific documentation. This work introduced the antioxidants of Liang leaves to create a new health-impact drinking product.

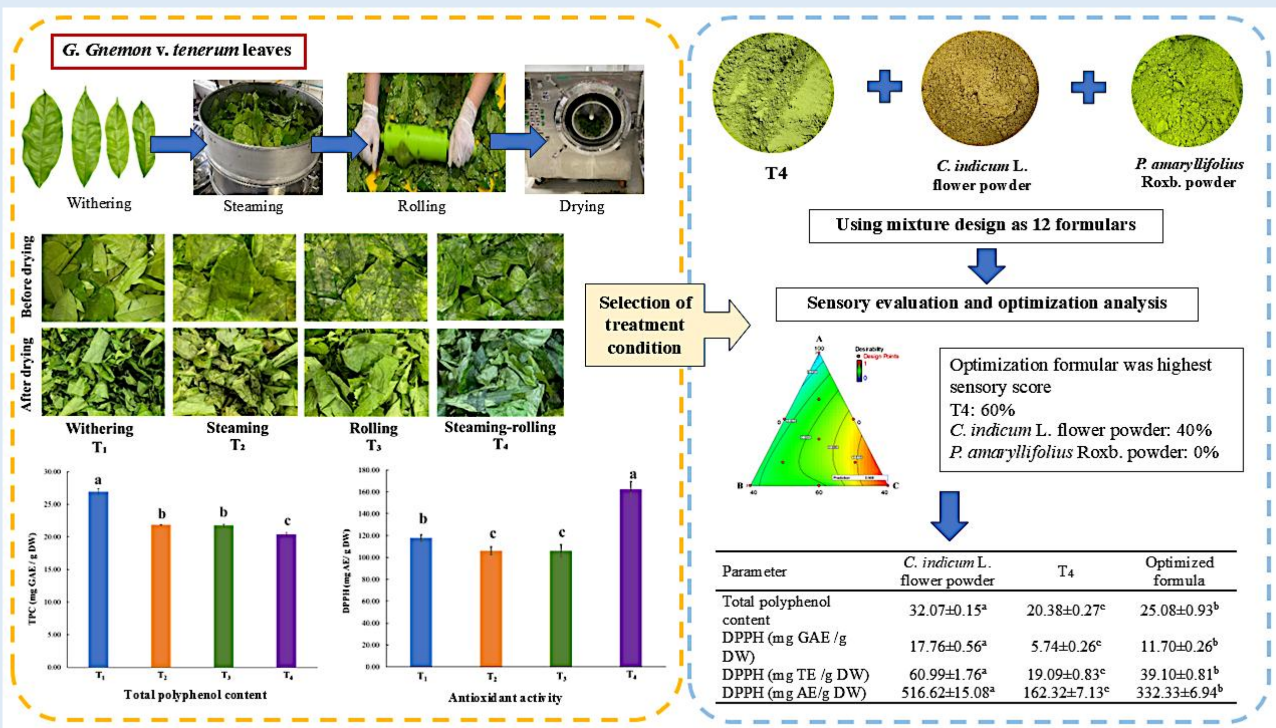
**Objective:** The research aimed to study the improvement of antioxidant activity and flavor using pretreatments, including withering, steaming, rolling, and steamed-rolling, and following the addition of fragrant plants.

**Method:** Liang leaves were pretreated by withering, steaming, rolling, and steaming rolling before drying. Then, the best condition was selected to add fragrant plants.

**Results:** Withering gave the highest total polyphenol content at 26.95±0.47 mg GAE/g DW compared with the other treatments ($p<0.05$), while higher DPPH scavenging activity was noticed in the steaming-rolling treatment ($p<0.05$). However, sensory evaluation results were not significantly different ($p>0.05$) in each pretreated tea. Steaming-rolling was selected based on the high antioxidant activity for further fragrance improvement using the powder of *Pandanus* *amaryllifolius* Roxb leaves and *Chrysanthemum indicum* L flowers at various ratios. The highest sensory score was recorded in tea made from a mixture of Liang leaf powder, *P. amaryllifolius* leaf powder, and *C. indicum* flower powder at a ratio of 60:0:40. The TPC and DPPH scavenging activities of the mixed tea powders were tested, with results of 25.08±0.93 mg GAE/g DW and 11.70±0.26 mg GAE/g DW, respectively. In addition, this product could be a good source of TPC and antioxidant activity judged by ascorbic acid as 332.33±6.94 mg AE / g DW.

**Conclusion:** The steaming-rolling treatment of Liang powder had the highest antioxidant activity, and then adding *Chrysanthemum indicum* L flowers powder at a ratio of 60:40 improved flavor, total polyphenol content, and antioxidant activity. The Thai indigenous vegetable, Liang leaves powder, is aimed for a novo alternative drink promoting health benefits.

**Keywords:** Liang leaves, Tea, Pretreatment, Flavor, Antioxidant activity




**Graphical Abstract:** Influence of pretreatment methods and addition of fragrant plants on *Gnetum gnemon* var t*enerum* powder tea



## INTRODUCTION

Tea is a widely consumed beverage known for its health benefits and diverse consumer appeal. Recently, tea products have become highly diverse and include white, green, oolong, black, hojicha, matcha, herbal, and flower tea. The variety of teas depends on plenty of raw materials, the creator, the culture of food consumption, and weather or climate conditions. For instance, Chinese and Japanese people regularly drink tea almost every meal, depending on the climate, temperature, or style of food consumption, as well as the ceremony. Drinking tea is deeply rooted in Chinese traditions, often passed down through generations, such as ginger tea, *Pandanus amaryllifolius* leaves tea, *Morus alba* leaves tea, and *Centella Asiatica* leaves tea.

In contrast, herbal teas must be served when people are sick or not in good condition. According to global connections by work, travel, and internet technology, food is spreading worldwide. Most people in each country learn, exchange, share, and modify their information, particularly food, fashion, folk medicine, etc. As known, Thailand is appointed as the world's kitchen due to the diversification of raw materials. However, processed food or products are still not as good as fresh conventional or sun-dried forms. Government and business sectors seek to focus more on creating more value-added ingredients and products. Therefore, several raw materials, particularly indigenous plants or organic ones, are intensively reviewed and appointed when nutritional values and specific functions are labeled or proven.

*Gnetum gnemon* var. *tenerum* (Liang) is a native vegetable shrub that produces small fruits grown in Southern Thailand [1]. The local people consume the apex, the young and intermediate Liang leaves (pae-slat), as a side dish, cooked in coconut soup or stir-fried with egg or an omelet [2]. *G. gnemon* var. *tenerum* leaves contained macronutrients, vitamins and minerals including carbohydrates 65.63±2.63 g/100g DW, proteins 25.41±0.68 g/100 g DW, total dietary fiber 41.27±0.16 g/100 g DW, vitamin A 3.03±0.07 mg/100g DW, vitamin C 2.71±0.04 mg/100g DW, calcium 450.71±5.37 mg/100g DW, copper 0.47±0.01 mg/100g DW, iron 3.63±0.04 mg/100g DW, magnesium 193.1±0.27 mg/100g DW, and zinc 3.18 mg/100g DW as well as high green pigment or chlorophyll content with 226.28± 22.25 mg/g DW together with complete essential amino acids and muscle enhancing compounds such as leucine, methionine, phenylalanine, lysine and tryptophan [3]. The *G. gnemon* var. *tenerum* leaves exhibited TPC and total flavonoid content (TFC) as 4.32 mg GAE/g DW and 1.4 mg quercetin equivalent/g DW, with high antioxidant activity [4]. The plant also showed antidiabetic properties [5] and gut microbiota enhancement [6]. According to the definition of bioactive compounds and functional foods, Liang leaves powder can be grouped as functional ingredients and foods based on bioactive compounds and their biological activities contained in the plant leaves related to promoted health benefits [7, 8]. The Thai government has recently promoted Liang as suitable for co-agriculture with other economic plants, including rubber, palm oil, durian, long gong, and other monopoly crops. Utilization of *G. gnemon* var. *tenerum* leaves in Thailand is not well commercialized. Still, there is projected to be a significant chance of investigation due to widespread cultivation and whole-year harvesting if adequately managed. So, Liang leaves tea was a product that added value, was low cost, was easy to process, and had health benefits. However, preliminary tests indicated that the plant leaves have undesirable flavor and some bitter taste when brewed as a tea drink.

As is well known, tea made from Camilla leaves has a unique flavor and aroma from withering, steaming, rolling, steamed-rolling, fermenting, and drying. The withering process involves moisture reduction to render soft leaves [9]. The steaming process stops the production of polyphenol oxidase enzyme and modifies tea's biological compounds, color, and volatile compounds [10]. The rolling process helps to generate the aroma, taste, and color due to plant cell breakdown, leading to more compound interactions and the creation and transformation of volatile compounds such as amino acids, fatty acids, carotenoids, and phenolics [11]. Steaming and rolling processes are usually advertent processes to improve tea quality.

*Pandanus amaryllifolius* Roxb leaves are widely used as natural flavoring and coloring agents in various drinks, desserts, bakery, and food products. In addition, *P. amaryllifolius* Roxb is intensively used for flavoring in desserts and several foods in Malaysia, India, the

Philippines, and Japan, as well as Thailand, such as cooking rice, pastries, sweet juice, and puddings [12]. Twenty-nine volatile compounds were identified in the leaves, including 2-acetyl-1-pyrroline, squalene, neophytadiene, and phytol as essential compounds giving distinctive aroma[13]. *Chrysanthemum indicum* L. flowers have been used to make commercial tea products in many countries because of their sweet scent, colorant, antioxidant, anti-inflammatory, and neuroprotective health functions [14]. The flowers contain many biological compounds, including polyphenols, flavonoids, carotenoids, anthocyanins, and terpenoids [15], with a strong aroma from more than 292 volatile compounds such as butanoic acid, 2-methyl-phenylmethyl ester, 2-propenoic acid and camphene [16].

Therefore, this study aimed to improve the flavor and antioxidant activities of *G. gnemon* var. *tenerum* powder by steaming and rolling pretreatment processes. To enhance its aroma and taste, the Liang leaf powder, after pretreatment, was mixed with powder of the fragrant plant *P. amaryllifolius* Roxb and *C. indicum* L. flower powder using a mixture design. Then, the sensory qualities, total polyphenol content (TPC), and antioxidant activities were investigated.

## MATERIALS AND METHODS

**Preparation of *Gnetum gnemon* var. *tenerum* leaves (Liang leaves):** Liang leaves (Figure 1a) were purchased from agricultural areas in Songkhla and Phatthalung Provinces. To control quality, Liang leaves were selected and used only in the intermediate stage (Pae-slat) by removal of the tip, young leaf, old leaf, and stem, as shown in Figure 1b. The loose leaves were cleaned with tap water and then soaked in 100 ppm chlorine solution for 15 min before rinsing twice with water to reduce the chlorine residue to less than 1 ppm as a safety condition.

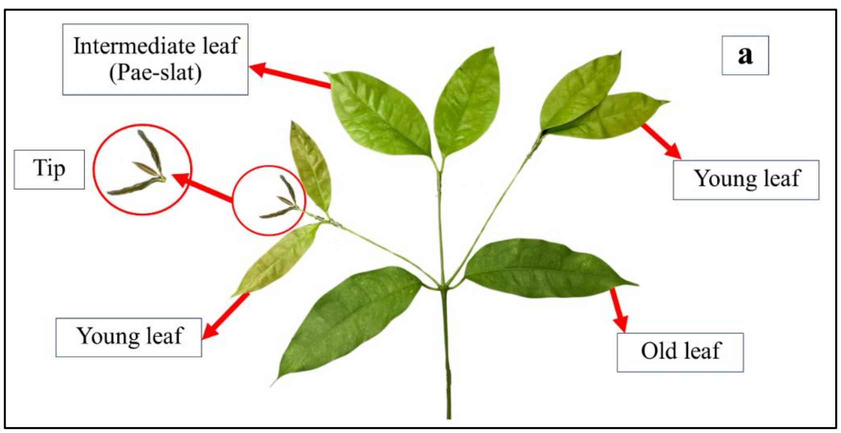




**Figure 1.** Stages of Liang leaves (a) and edible stage (Pae-slat) used in this experiment (b)

**Preparation of *P. amaryllifolius* Roxb. and *C. indicum* L. powder:** *P. amaryllifolius* Roxb. leaves were purchased from a farmer in Phatthalung Province, cleaned, and soaked in 100 ppm chlorine solution and then rinsed twice with tap water. As a conventional method or control sample, the leaves were cut and dried in a hot air oven at 60 °C for 12 h. Due to the limitation for wet sample blending, the leaves were blended with drinking water at 3:5 to make a slurry and then dried with drum dry at 120-125 °C for 1.5 min to obtain moisture less than 8%. The dry sample was ground using a high-speed grinder and screened by sieve no. 230 with a mesh size of 63 µm. Both conventional and drum-dried samples were taken for sensorial evaluation using focus group persons for drying method selection. The focus group result suggested that using a hot air oven caused a loss of more fragrant flavor. Therefore, the sample obtained from the drum dryer was selected and used further.

Dried *C. indicum* L. flowers in a sealed package and certified by the Thai FDA were purchased from Superstore in Thailand. The dried sample was washed and soaked using a procedure similar to the Liang and *P. amaryllifolius* preparation. Still, it was rinsed four times with tap water to remove chlorine residue and smell. The cleaned sample was then dried in a hot air oven at 60 °C for 12 h to attain a moisture content of 8% before grinding in a high-speed grinder and screening through sieve no. 230 with a mesh size of 63 µm.

**Pretreatment process of *G. gnemon* var. *tenerum* leaves**: The fresh Liang leaves were divided into four groups as Treatment 1 ($T_{1)}$; Liang leaves were spread on a steel tray and withered at 25-27 °C for 24 h. Treatment 2 (T2): Liang leaves were first withered and then steamed with boiling water for 1 min. Treatment 3 ($T_3$): Liang leaves were first withered and then rolled using a rolling pin for 15 min. Treatment 4 ($T_4$): Liang leaves were first withered before steaming with boiling water for 1 min and rolled for 15 min. A flowchart protocol of sample preparation is depicted in Figure 2. All treatments were subjected to microwave vacuum drying at 3600 watts for 20 min following the protocol of Suksang *et al*. (2023) [5]. The dried sample was finely ground using a high-speed grinder and sieved through a no. 230 mesh with a size of 63 µm, and stored in aluminum foil packaging at -20°C. Moisture content, $a_w$, pH, solubility, TPC, antioxidant activity, and sensory acceptability were evaluated, and the optimal condition for aroma and taste improvement was selected in the next step by adding *P. amaryllifolius* leaf powder and *C. indicum* flower powder.

**Optimization of the powder of *G. gnemon* var. *tenerum* leaf, *P. amaryllifolius* leaf, and *C. indicum* L flower:** Mixtures of Liang leaf powder, *P. amaryllifolius* leaf powder, and *C. indicum* L. flower powder was established according to an augmented simplex-centroid design. Proportions of Liang leaf powder (60-100%), *P. amaryllifolius* leaf powder (0-40%), and *C. indicum* L. flower powder (0-40%) were used, resulting in 12 design points as 10 treatments with 2 replications, as shown in Table 1. All samples were taken for sensory evaluation, and one formula with the highest consumer acceptance was selected with optimization analysis by Desing-Expert 13. Then, the chosen formula was measured for moisture content, $a_w$, pH, solubility, total polyphenol compounds, and antioxidant activity.

**Table 1.** Formula designs for Liang drink mixed with fragrant plants, *P. amaryllifolius* leaf powder, and *C. indicum* L. flower powder.

| Formula | Liang leaf powder (%) | *P. amaryllifolius* leaf powder (%) | *C. indicum* L. flower powder (%) |
|---|---|---|---|
| **F1** | 86 | 7 | 7 |
| **F2** | 74 | 13 | 13 |
| **F3** | 60 | 20 | 20 |
| **F4** | 60 | 0 | 40 |
| **F5** | 80 | 20 | 0 |
| **F6** | 67 | 27 | 6 |
| **F7** | 67 | 6 | 27 |
| **F8** | 60 | 40 | 0 |
| **F9** | 100 | 0 | 0 |
| ***F10** | 60 | 40 | 0 |
| ***F11** | 100 | 0 | 0 |
| **F12** | 80 | 0 | 20 |

*Replications

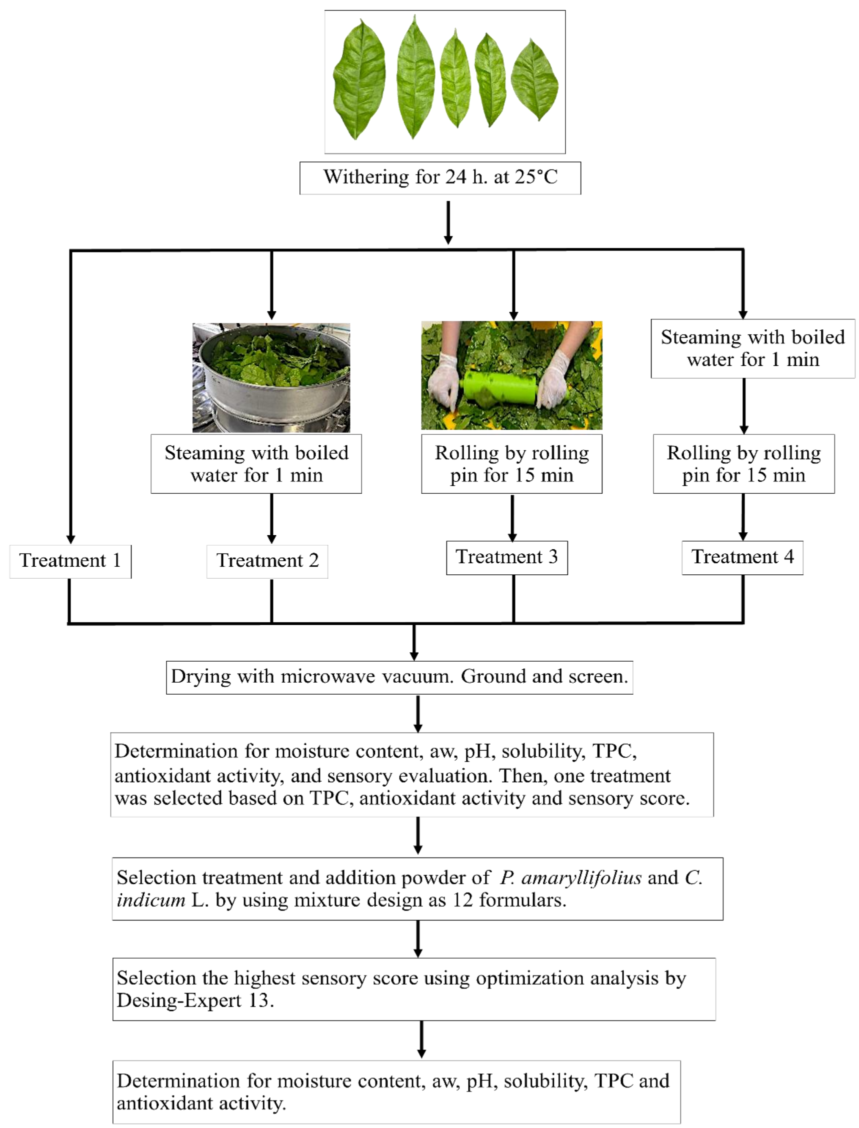



**Figure 2.** Diagram of experiment.

**Preparation of sample extracts:** Each mixed-dried sample was extracted with hot water (95-99 °C) at a ratio of 1:20 (sample: water) with stirring condition for 5 min, following the method of Srisook *et al.* (2021) [17] with some modifications. The samples were separated by vacuum suction using a Buchner funnel before centrifuging at 4°C for 20 min at 8,000 rpm (CR22G III, Hitachi Koki Co., Ltd., Hitachinaka City, Japan). The clear supernatant was freeze-dried, and the powder was stored at -20°C until use.

**Physicochemical properties determination**

**Moisture content:** According to AOAC, 2019 [18], Moisture content was determined. An empty moisture can and lid were dried in an oven at 105 °C for 3 h to complete dryness and transferred to a desiccator to cool before weighing. Three grams of the sample were placed in the dish and spread uniformly before drying in the oven at 105 °C until constant weight. The dish containing the dry sample was partially covered with the lid and placed in the desiccator to cool before weighing.

**Water activity ($a_w$) value:** Water activity was determined using a water activity meter (Meter Group, Inc. USA).

**pH:** One gram of the sample was well blended with distilled water at a ratio of 1:150, and the pH was determined using a pH meter (Sartorius AG, Docu-pH+ Meter, Göttingen, Germany).

**Water solubility:** With modifications, water solubility was determined, as described by Shittu and Lawal (2007) [19]. Briefly, 1 g of each sample was mixed in 150 ml of boiled water for 15 min, stirring before filtering using Whatman no.1 filter paper and a Buchner vacuum pump. The supernatant was dried in a hot air oven at 105 °C for 24 h. The weight of the solid after drying was used to calculate the water solubility as follows:

$$\%\boldsymbol{Solubility} = \left[\frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_2}}\right] \times \mathbf{100}$$

$W_1$: weight before drying; $W_2$: weight after drying

**Total polyphenol content (TPC):** The TPC was determined using the method described by Suksanga *et al*. (2023) [20] with some modifications. Each sample extract was weighed and diluted with distilled water to give an optical density (O.D.) of absorbance at 0.2-0.8. Then, 20 µl of the sample extract was added to 96-well plates, followed by 100 µl of 10% Folin reagent (v/v). After incubation in the dark at 30 °C for 6 min, 7.5% $Na_2CO_3$ (anhydrous) (w/v) was added, and the mixture was incubated for another 30 min. The absorbance was measured at 765 nm using a microplate reader (Varioskan LUX, Thermo Scientific, Singapore). TPC content was reported as mg gallic acid equivalent (GAE)/g dry weight (DW) using gallic acid as the standard at a concentration of 0-80 µg/ml ($R^2$ = 0.996).

**Antioxidant activity measurement by DPPH radical scavenging assay:** The 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity was determined using the method described by Suksanga *et al*. (2023) [20] with some modifications. Each sample extract was weighed and diluted with distilled water to give an optical density (O.D.) of absorbance at 0.2-0.8. Then, 100 µl of the sample extract was mixed with 100 µl of 0.2 mM DPPH in 95% ethanol. The sample was incubated in the dark for 30 min at 30 °C. Finally, the absorbance of the mixture was measured at 517 nm and reported as µg gallic acid, Trolox (TE), and ascorbic acid (AE) equivalent/g DW. Gallic acid, Trolox, and ascorbic acid were used as the standards at a concentration of 0-3.5 µg/ml ($R^2$ = 0.9993), 0-14 µg/ml ($R^2$ = 0.9958), and 0-120 µg/ml ($R^2$ = 0.995), respectively.

**Sensory evaluation:** The sensory evaluation used a 9-point hedonic scale for acceptability (%). The effects of pretreatments on Liang leaf powder tea were evaluated. The optimization of mixed powder ratios of Liang leaf powder and fragrant plants was conducted by 60 untrained panelists aged 18 to 30. Approximately 1 g of the sample was stirred with boiled water (ratio 1:150) and served at 70 °C in a shot glass. Each sample was coded randomly with three digits. The seven characteristics of the 9-point hedonic scale included appearance, color, aroma, taste, bitterness, aftertaste, and overall liking. Plain drinking water at room temperature (25-27 °C) was provided for mouth rinsing between samples. Thereafter, panelists were further asked for acceptability or unacceptability for each sample before being taken to percentage acceptability.

**Statistical analysis:** A completely randomized design (CRD) was followed for the pretreatment of Liang leaf powder tea. At the same time, sensory testing of Liang drink mixed with fragrant plants, *P. amaryllifolius* and *C. indicum* L., used a randomized complete block design (RCBD). The data were assessed by a one-way analysis of variance (ANOVA), with mean comparisons analyzed by Duncan's new multiple range test (DMRT) at a significant level of 0.05 using SPSS Statistics 22 (SPSS Inc., IBM, NY, USA) software. Optimization was analyzed by Desing-Expert 13 (Design-Expert® software, Stat-Ease, Inc., Minneapolis, MN, USA).

## RESULTS AND DISCUSSION

### Effect of pretreatments on the physicochemical properties, TPC, antioxidant activity, and sensory evaluation of Liang leaf powder tea

**Physicochemical properties:** The moisture contents of Liang leaf powder from $T_1$, $T_2$, $T_3$, and $T_4$ pretreatments were 4.28±0.00%, 5.61±0.08%, 5.30±0.37%, and 5.71±0.33%, respectively (Table 2). The $T_1$ pretreatment gave the lowest moisture content compared with the others ($p<0.05$). Interestingly, the $a_w$ value of $T_4$ was the lowest (0.26±0.01) even though it contained higher moisture content. The non-alignment between moisture content and $a_w$ in T1 and T4 was due to cell disruption in T4 (steamed rolling), which promoted the drying process but also released more minerals, including Na, K, Mg, P, Ca, etc., which accelerated $H_2O$ reabsorption, leading to higher moisture content. After the powdering step, the moisture content of T4 increased. Xie *et al*. (2023) [21] reported that steamed *Gastrodia elata* plants suffered more cell wall rupture, soft tissue, and water loss due to destroyed pectin. Steaming carrots induced swelling of the parenchyma and increased plasmolysis, while rolling or threshing broke down the leaf structure, and the reduced moisture led to wrinkles [22].

The pH values of $T_1$, $T_2$, $T_3$, and $T_4$ were 6.03±0.05, 6.11±0.00, 6.11±0.02, and 6.11±0.02. $T_1$ exhibited a lower pH than the other treatments ($p<0.05$), with no harsh processes during pretreatment and drying. Thus, cell structures were not damaged without phytochemicals as weak acids such as vitamin C, phenolic acid, and minerals. High heat treatment (70-90 ºC) for 15-120 s reduced vitamin C in orange juice following a first-order reaction [23]. Vegetables, including carrots, chard, potato, and sweet potatoes, under the steamed process showed vitamin C content reduction compared with the fresh form. However, some vegetables, such as broccoli and zucchini, indicate an increment of vitamin C after the heating process ($p<0.05$) [24]. Herbal tea from steamed-blanched *Moringa oleifera* leaves showed higher pH than unblanched leaves [25].

The $T_3$ pretreatment had the highest water solubility ($p<0.05$), followed by $T_4$, $T_2$, and $T_1$, respectively, as shown in Table 1, because the rolling process broke down the cell wall structure and allowed water to contact with cell organelles, with free molecules either released or repelled. Without preheated treatment, labile molecules were more preserved. Results indicated that soluble dietary fiber, protein, sugars, minerals, and vitamins showed more solvation during physical pretreatment, including heating, pressing, and blending. Steaming *Telfairia occidentalis* leaves with boiling water for 5 minutes reduced nitrogen, phosphorus, potassium, sodium, calcium, magnesium, and iron contents compared with fresh leaves [26].

Samples with lower pH showed a reduced water solubility, while pH responded to acid and basic content and buffering capacity. Food containing more acid with less buffering capacity, such as citrus fruits, had a low pH value. Still, if the buffering capacity is high, then the pH of the sample, such as tuber or leafy plants, also increases. Lactic fermented meat with high buffering capacity did not have a low pH value, as found in meat with less buffering capacity. Each parameter impacted the others in diverse ways.

**Table 2**. Moisture content, water activity, pH, and pretreated var. *tenerum* leaf powder solubility.

| Pretreatment | Moisture (dried leaves before powdering) (%) | Moisture (dried leaves after powdering) (%) | Water activity ($a_w$) | pH | Water solubility (%) |
|---|---|---|---|---|---|
| $T_1$ | 4.57±0.03 $^{a}$ | 4.28±0.00$^{a}$ | 0.36±0.01$^{b}$ | 6.03±0.05$^{a}$ | 65.11±0.05$^{c}$ |
| $T_2$ | 5.67± 0.01$^{b}$ | 5.61±0.08$^{b}$ | 0.39±0.04$^{b}$ | 6.11±0.00$^{b}$ | 65.78±0.54$^{bc}$ |
| $T_3$ | 5.40±0.26 $^{b}$ | 5.30±0.37$^{b}$ | 0.38±0.01$^{b}$ | 6.11±0.02$^{b}$ | 68.22±0.73$^{a}$ |
| $T_4$ | 5.31±0.31$^{b}$ | 5.71±0.33$^{b}$ | 0.26±0.01$^{a}$ | 6.11±0.02$^{b}$ | 66.09±0.37$^{b}$ |

n= 3; Mean±SD. Values in a column followed by different superscripts are significantly different (p<0.05). $T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4.

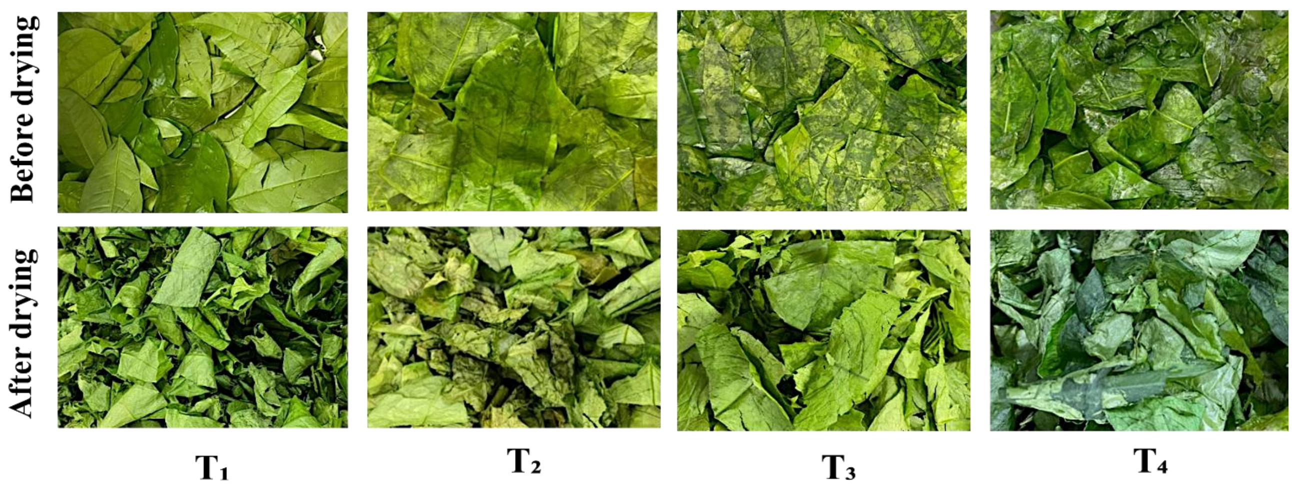



**Figure 3**. Pretreatment of var. *tenerum* leaves before and after microwave drying. $T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4.

**Total polyphenol content:** The TPC of var. *tenerum* leaf powder after $T_1$, $T_2$, $T_3$, and $T_4$ pretreatments were 26.95±0.47, 21.84±0.05, 21.76±0.19 and 20.38±0.27 mg GAE/g DW (Figure 4A). $T_1$ provided the highest TPC ($p<0.05$), while $T_2$ and $T_3$ were not significantly different ($p>0.05$), and $T_4$ was the lowest. Every action, including heating, provides positive and negative effects to weaken the cell structure, thereby facilitating extraction and destroying some heat-sensitive compounds. Temperature was an essential factor for TPC degradation, with 70-90 °C showing a decreasing trend following a first-order reaction [27]. Precooking by steaming *Moringa oleifera* leaves and drying showed lower TPC values than unblanched leaves [25]. Using more force during rolling liberated phenolic compounds from the plant cell walls because of cell damage and disappeared with water loss. Phenolic acid generated during the rolling process is oxidized by polyphenol oxidase enzyme (PPO), which occurs in plastids of plant cells and is secreted when leaves are damaged. TPC of rolled leaves of *Camellia sinensis* L. was lower than fresh leaves ($p<0.05$) during black tea processing but not different from the withering process ($p>0.05$) [28]. Zhang *et al*. (2023) [29] reported that *C. sinensis* L. leaves subjected to the rolling process showed reduced flavan-3-ols and their derivatives, flavanol glycosides, and phenolic acids. Coffee leaf tea was divided into five steps: fresh leaves, withering, rolling, fermenting, and drying. Rolled leaves gave lower free phenolic content due to the leaching effect and/or oxidation stress function compared with the withering step; however, bound phenolic content was higher compared with the withering step ($p<0.05$) [30]. Some phenolics, including *p-coumaric* and ferulic acids, degraded during the drying process, with red quinoa and white quinoa leaves at 60°C yielding the lowest TPC followed by 45 and 30 °C [31]. This result indicated that the TPC of quinoa leaves was temperature dependent. The higher the temperature, the lower the TPC retained. Results implied that the TPC contained in the quinoa consisted of heat-sensitive compounds. Vacuum-microwave drying of *Prunus cerasus* L. (sour cherries) revealed a more significant loss of polyphenol compounds than fresh leaves [32]. More than 206, 41, and 35 compounds found in *Camellia sinensis* leaves tea were changed by steaming, rolling, and drying [33]. It pointed out that the processing step may cause a significant change, particularly with harsh conditions (high temperature, long time, high force). However, using the Folin-Ciocalteu reagent is not a selective method because it can react with non-phenolics and with other compounds, including vitamin C, thiamin, folinic acid, retinoic acid, cysteine, and tyrosine [34]. Among the 4 formulas, TPC in T1 was higher than others, possibly due to more vitamin C and/or polyphenolics due to lesser harsh conditions.

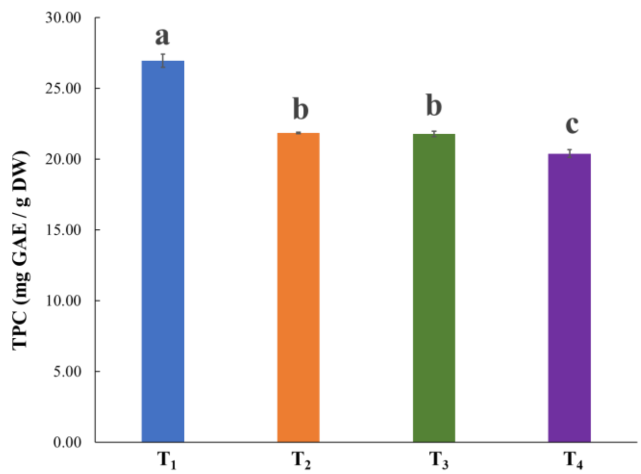



**Figure 4.** Total polyphenol content (TPC) of var. *tenerum* leaf powder after pretreatment. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). $T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4. Error bars show mean ± standard deviation from four repeats. GAE = gallic acid equivalent. DW = dry weight.

**Antioxidant activity:** Antioxidant activity was assessed using the DPPH radical scavenging method, referencing three standards: gallic acid, Trolox, and ascorbic acid, as shown in Table 3. The result indicated that T 4 provided the highest DPPH activity at 5.74±0.26 mg GAE/g DW, followed by T2 (3.78±0.14 GAE/g DW), T3 (3.73±0.21 GAE/g DW) > T1 (4.17±0.11 GAE/g DW). Based on standard Trolox, the highest antioxidant activity was also found in T 4, which was higher than using GAE about 3-4 times. In addition, this powder provided antioxidant activity based on ascorbic acid higher than the 105 mg/g sample, which was enough for the body of recommended value as 95-110 mg/d (Thai RDI) [35]. Antioxidant activity showed different values when using different standard equivalents. Gallic acid was the lowest number because of its small molecule and contains 3 hydroxy groups, indicating a robust transfer free radical. This result was in accordance with the findings of Hwang and Lee (2023)

[36], who report that using gallic acid as standard in DPPH assayed was the lowest value, followed by catechin, ascorbic acid, and Trolox. In addition, antioxidant activity using the ascorbic acid equivalent of Liang tea (> 105 mg AE/g DW) was higher than apple at 49.08 ± 6.08 mg AE / g DW. It pointed out that taking Liang tea gained more health benefits if determined as having ascorbic value and without or less energy load due to the lack of sugar in raw materials or samples.

The $T_4$ formula gave the highest DPPH assay ($p<0.05$), followed by $T_1$, $T_2$, and $T_3$. As mentioned earlier, $T_4$ helped to weaken leaf cell walls with increased porosity, leading to higher extractability. When plant cells are injured or damaged, oxidative stress produces free radicals and antioxidants to combat the radicals. The antioxidant activity indicates the ultimate value of creating and using antioxidant compounds in each plant. Therefore, a higher antioxidant activity means production is more significant than consumption. At the same time, a lower value in any tested plant indicates that using or degrading is more substantial due to serious factors such as microbial invasion, drought, high temperature, etc. [37]. The blanching of *Cinnamomum porrectum* leaves showed a lower antioxidant activity than fresh ones when assessed with the DPPH scavenging method. However, 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) activity, ferric reducing antioxidant power (FRAP), and iron chelating of blanched samples were higher than unblanched ones [38]. However, antioxidant activities may not align with TPC, but the quality or type of polyphenol compounds are also impacted and matched by different methods. For example, the antioxidant activities of L-ascorbic acid, catechin, quercetin, sesamol, ferulic acid, gallic acid, morin, and d-alpha-tocopherol were 1,080, 2,430, 4,290, 980, 780, 2,810, 990 and 900 mmolTE/mol, respectively. Each phenolic compound exhibited different potentials for antioxidant activity [39]. These results concurred with Wickramasinghe *et al*. (2020) [25], who reported that the DPPH scavenging capacity of steamed-blanched *M. oleifera* leaves was higher, while TPC was lower than in the unpretreated leaves. Thus, every action mode had both pros and cons.

**Table 3**. Antioxidant activity by DPPH radical scavenging assay of var. *tenerum* leaf powder after pretreatment.

| **Treatments** | **Antioxidant activity** | | |
|---|---|---|---|
| | mg GAE /g DW | mg TE /g DW | mg AE / g DW |
| **$T_1$** | 4.17±0.11b | 13.86±0.34b | 117.85±2.95b |
| **$T_2$** | 3.78±0.14c | 12.48±0.44c | 106.19±3.74c |
| **$T_3$** | 3.73±0.21c | 12.47±0.68c | 105.98±5.79c |
| **$T_4$** | 5.74±0.26a | 19.09±0.83a | 162.32±7.13a |

Different lowercase letters indicate significant differences between treatments ($p < 0.05$). $T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4. Error bars show mean ± standard deviation from four repeats. GAE = gallic acid equivalent. TE = Trolox equivalent. AE = ascorbic acid equivalent. DW = dry weight.

**Sensory evaluation:** Sensory evaluation of var. *tenerum* tea, including appearance, color, flavor, taste, bitterness, aftertaste, and overall liking, are shown in Table 4. Results indicated that pretreatment did not significantly improve the sensory perception of Liang leaves powder tea. The panelists also noted an undesirable flavor and taste. Resulting in a low acceptability score of less than 95%. Observations and results of the focus group indicated that the tea had an unpleasant flavor with a fishy-like smell, and its reasons were unclear. However, the high protein content of 25.41±0.68 g/100g DW with 17 types of amino acids in Liang leaves [3] may be suspected substances of unpleasant flavor like meaty- fishy smell initiators even other compounds may be involved such as short chain fatty acid (butyric acid) and aldehydes such as hexanal, heptanal, 2,4-heptadienal, 1-octen-3-one, 1-octen-3-ol, octanal, 2-octenal, 2,4-octadienal, nonanal, 2-nonenal, 2,6-nonadienal, decanal, 2-decenal, 2,4-decadienal, undecanal, 2-tetradecanone [40] or polyunsaturated aldehydes metabolites of lipid and polyunsaturated fatty acids (PUFAs) [41]. Laska (2010) [42] stated that 6 amino acids, including L-cysteine, D-cysteine, L-methionine, D-methionine, L-proline, and D-proline played key roles in food off-flavors. Cysteine had a sulfur and rotten eggs odor, while methionine was described as moldy, old potato, and rotten dairy products. Proline had a semen, sperm, and chlorine-like smell. Human thresholds for cysteine, methionine, and proline concentration were 0.2-0.22, 0.01-0.08, and 75-100 mM, respectively. *G. gnemon* var. *tenerum* leaf powder contained cysteine, methionine, and proline at 0.06 (8.22mM), 0.36 (32.33 mM) and 1.33 (161.73mM) g/100g DW (unpublished data) giving off-flavors and impacting panelist acceptability. However, cysteine, methionine, and proline are beneficial in the human body. Cysteine synthesizes protein and supports enzyme catalysis, especially L-cysteine, which was reported for supplements with pharmacological activity, including antioxidants, improved immune system, prevent heart disease, and strengthen hair [43]. Methionine is a precursor in the metabolism of homocysteine, succinyl-CoA, cysteine, creatin, and carnitine and plays an essential role in glutathione promotion, leading to boosting the immune system and reduction of oxidative stress [44]. Proline supports major protein and collagen synthesis and cell regulation through signaling molecules, cell proliferation, and differentiation [45]. The sensory scores showed that tea made from $T_4$ was better accepted with higher scores (≥6) given by 50% of the panelists, as shown in Table 5. Taking antioxidant activity to comply with sensory acceptability led to $T_4$ selection for optimization added with fragrant plants.

**Table 4**. Sensory evaluation of pretreatments of *G. gnemon* var. *tenerum* leaf powder tea.

| Attribute | $T_1$ | $T_2$ | $T_3$ | $T_4$ | ANOVA |
|---|---|---|---|---|---|
| **Appearance** | 6.52±1.44 | 6.67±1.40 | 6.60±1.55 | 6.45±1.32 | ns |
| **Color** | 6.78±1.53 | 6.78±1.37 | 6.90±1.47 | 6.68±1.62 | ns |
| **Flavor** | 5.33±1.67 | 5.75±1.45 | 5.62±1.75 | 5.43±1.70 | ns |
| **Taste** | 5.40±1.65 | 5.67±1.65 | 5.80±1.88 | 5.43±1.67 | ns |
| **Bitterness** | 6.13±1.50 | 6.07±1.72 | 6.05±1.84 | 6.02±1.86 | ns |
| **Aftertaste** | 5.87±1.63 | 5.75±1.61 | 5.72±1.91 | 5.50±1.87 | ns |
| **Overall liking** | 6.18±1.37 | 6.08±1.38 | 6.03±1.65 | 5.93±1.58 | ns |

Mean±SD. Ns; not significant with p>0.05. $T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4.

**Table 5**. Percentage of panelists who gave scores greater than 6 scores in each attribute

| Attributed | Percentage of panelist (%) | | | |
|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| **Appearance** | 78.33 | 80 | 80 | 81.67 |
| **Color** | 81.67 | 83.33 | 80 | 85 |
| **Flavor** | 51.67 | 63.33 | 48.33 | 53.33 |
| **Taste** | 46.67 | 55 | 45 | 58.33 |
| **Bitterness** | 66.67 | 60 | 60 | 70 |
| **Aftertaste** | 60 | 55 | 46.67 | 56.67 |
| **Overall liking** | 70 | 65 | 65 | 66.67 |

$T_1$: Treatment 1; $T_2$: Treatment 2; $T_3$: Treatment 3; $T_4$: Treatment 4.

**Sensory evaluation and optimization of mixed tea powders of G. *gnemon* var. *tenerum* leaves, *P. amaryllifolius* Roxb leaves, and *C. indicum* L. flowers:** Previous results revealed that teas made from var. *tenerum* leaf powder did not meet consumer preference. However, after adding *P. amaryllifolius* leaf and *C. indicum* L. flower powders, the drink was more acceptable at 78%. Results indicated that F4 containing 60% *G. gnemon* var.

*tenerum* leaf powder and 40% *C. indicum* L. flower powder obtained higher flavor, taste, bitterness, aftertaste, and overall liking scores, as shown in Table 6. The sensory score of F4 was not significantly different from F7. However, every sensory attribute of F4 was higher than 6 on the 9-point hedonic scale, indicating higher consumer acceptability, while the other formulas recorded lower than 6 points for at least 2 attributes. Adding the flower powder to the Liang tea gave higher sensory scores than adding *P. amaryllifolius* leaves powder because of the grassy and green odor aftertaste. The sensory results confirmed that using only Liang leaf powder (F9 and F11) gave the lowest acceptability scores compared with the formulas added with fragrant plants, as expected.

A regression model was used to analyze the relationship between the proportions of *G. gnemon* var. *tenerum* leaf powder, *P. amaryllifolius* leaf powder, and *C. indicum* L. flower powder on sensory attribute scores (appearance, color, flavor, taste, bitterness, aftertaste, and overall liking). A regression model was also used to predict the sensory attribute scores when the mixture proportions changed [46]. However, the regression model of the bitter taste attribute could not be used to predict sensory scores because the R-squared ($R^2$) value was lower than 0.75. The model of appearance could also not be used because the probability of the model was non-significant ($p>0.05$). The lack of fit in the regression model of flavor was significant ($p<0.05$), indicating that the model did not fit the data. However, the models of color, taste, aftertaste, and overall liking attributes were valid for Liang tea (Table 7). Results indicated that the F4 formula was like the optimization score ($p>0.05$), with the predicted scores of color, taste, aftertaste, and overall liking as 6.65, 6.47, 6.07, and 6.39, respectively. The desirability values of the formulas ranged from 0 to 1, which near 1 refers to the optimal condition [47]. Prediction of the formulas gave desirability as 0.949, as shown in Figure 5a.

Adding more *C. indicum* into the Liang powder enhanced a higher sensory score after making a tea because of its fragrant compounds such as 4-isopropyl toluene, camphoraceous, (E, E)-2,4-octadienal, caryophyllene and borneol [48] which were liberated from the flowers during hot soaking. Previous research found seven major groups in *C. indicum,* including camphor (minty and like eucalyptus), borneol (menthol, herbaceous, and woody), verbenol (herbal), endobornyl acetate, terpinene-3-ol (woody, piney and light musty), α-amorphene, and selinene (citrusy, fresh, herbal, and woody) [49]. The volatile compounds of *C. indicum* L. masked the unpleasant flavor of *G. gnemon* var. *tenerum* leaf powder.

**Table 6.** Sensory evaluation of var. *tenerum* leaf powder tea added with *P. amaryllifolius* Roxb leaf powder and *C. indicum* L. flower powder.

| Formula | Attributed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Appearance | Color | Flavor | Taste | Bitterness | Aftertaste | Overall liking | Acceptance |
| **F1** | $6.40±1.48^{ns}$ | $6.65±1.28^{ns}$ | $5.19±1.98^{cd}$ | $5.73±1.86^{abcd}$ | $6.04±1.78^{ab}$ | $5.52±1.94^{abcd}$ | $5.67±1.71^{bcd}$ | 59.62% |
| **F2** | $6.44±1.60^{ns}$ | $6.62±1.50^{ns}$ | $5.73±1.79^{bc}$ | $5.88±1.65^{abc}$ | $6.04±1.85^{ab}$ | $5.71±1.75^{abc}$ | $5.92±1.64^{abc}$ | 71.15% |
| **F3** | $6.13±1.73^{ns}$ | $6.23±1.67^{ns}$ | $6.17±1.83^{ab}$ | $5.85±1.96^{abc}$ | $5.90±1.95^{ab}$ | $6.00±1.90^{ab}$ | $6.02±1.76^{ab}$ | 71.15% |
| **F4** | $6.25±1.65^{ns}$ | $6.65±1.25^{ns}$ | $6.23±1.92^{a}$ | $6.21±1.75^{a}$ | $6.25±1.80^{a}$ | $6.08±1.97^{a}$ | $6.38±1.63^{a}$ | 78.85% |
| **F5** | $6.19±1.58^{ns}$ | $6.73±1.42^{ns}$ | $5.10±1.71^{d}$ | $5.15±1.86^{def}$ | $5.38±1.87^{bc}$ | $5.21±1.89^{cdef}$ | $5.25±1.89^{de}$ | 50.00% |
| **F6** | $6.17±1.74^{ns}$ | $6.54±1.57^{ns}$ | $5.58±1.88^{bcd}$ | $5.23±2.22^{cdef}$ | $5.40±2.18^{bc}$ | $5.40±2.29^{bcde}$ | $5.50±2.08^{bcde}$ | 59.62% |
| **F7** | $6.21±1.61^{ns}$ | $6.52±1.45^{ns}$ | $5.94±1.93^{ab}$ | $6.17±1.99^{a}$ | $6.21±1.89^{a}$ | $5.98±2.11^{ab}$ | $6.29±1.84^{a}$ | 71.15% |
| **F8** | $6.35±1.49^{ns}$ | $6.56±1.34^{ns}$ | $5.71±2.11^{abc}$ | $5.44±2.20^{bcde}$ | $5.27±2.39^{c}$ | $5.37±2.14^{bcde}$ | $5.40±2.18^{cde}$ | 53.85% |
| **F9** | $6.44±1.49^{ns}$ | $6.77±1.49^{ns}$ | $5.15±1.84^{cd}$ | $4.96±2.10^{ef}$ | $5.23±2.01^{c}$ | $4.81±2.02^{ef}$ | $5.04±1.99^{e}$ | 42.31% |
| **F10** | $6.29±1.54^{ns}$ | $6.52±1.35^{ns}$ | $5.73±1.83^{abc}$ | $5.27±1.88^{cdef}$ | $4.94±2.13^{c}$ | $5.04±1.83^{def}$ | $5.23±1.84^{de}$ | 53.85% |
| **F11** | $6.31±1.49^{ns}$ | $6.62±1.35^{ns}$ | $5.12±1.68^{d}$ | $4.73±1.65^{f}$ | $5.00±1.91^{c}$ | $4.73±1.95^{f}$ | $5.13±1.66^{de}$ | 44.23% |
| **F12** | $6.42±1.46^{ns}$ | $6.54±1.51^{ns}$ | $6.04±1.64^{ab}$ | $5.96±1.87^{ab}$ | $6.04±2.01^{ab}$ | $5.83±1.85^{abc}$ | $5.96±1.79^{abc}$ | 69.23% |

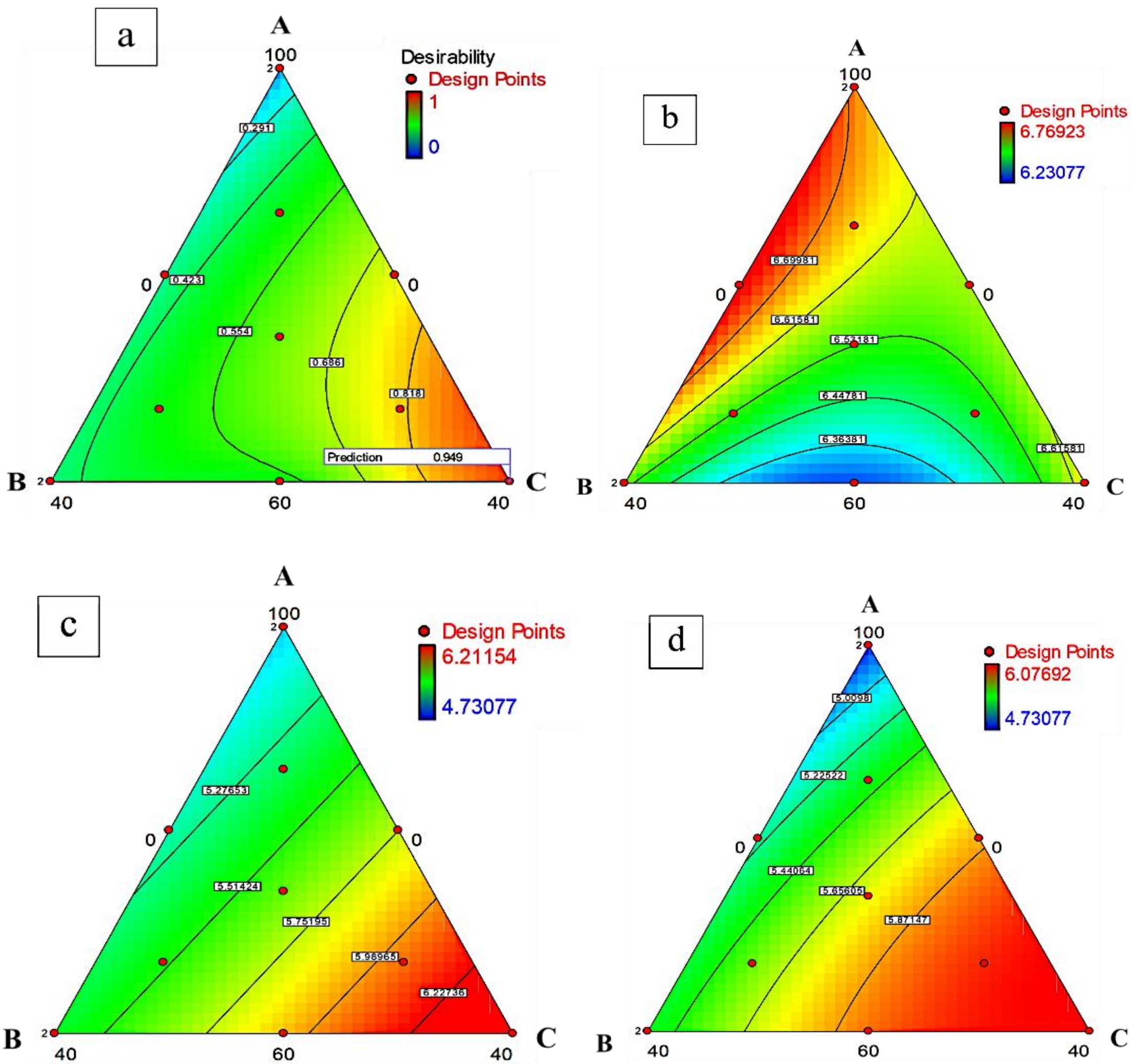
Mean±SD. values in a column followed by different superscripts are significantly different ($p<0.05$). ns; not significant at $p>0.05$. $T_1$: Withering treatment; $T_2$: Steaming treatment; $T_3$: Rolling treatment; $T_4$: Steamed-rolling treatment.

**Table 7.** Regression models of var. *tenerum* leaf powder tea added with *p. amaryllifolius* Roxb leaf powder and *C. indicum* L. flower powder.

| Characteristics | Regression model | $R^2$ | Probability of model | Lack of fit |
|---|---|---|---|---|
| **Appearance** | Y= 0.063835A + 0.088682B + 0.043552C-4.34123x$10^{-4}$AB + 2.72418x$10^{-4}$AC -4.50625x$10^{-3}$BC + 6.74086x$10^{-5}$ABC | 0.75 | 0.1630 | 0.3763 |
| **Color** | Y= 0.066858A + 0.039907B + 0.077541C + 3.93780x$10^{-4}$AB | 0.87 | 0.0136 | 0.6817 |
| **Flavor** | Y= 0.051228A + 0.12619B + 0.040737C-9.98943x$10^{-4}$AB + 6.28407x$10^{-4}$AC + 3.70017x$10^{-4}$BC | 0.96 | 0.0005 | 0.0183 |
| **Taste** | Y= 0.050388A + 0.059412B + 0.086044C | 0.79 | 0.0008 | 0.1672 |
| **Bitter** | Y= 0.053588A + 0.052986B + 0.082437C | 0.74 | 0.0025 | 0.1508 |
| **Aftertaste** | Y= 0.047944A + 0.043178B + 0.017964C + 3.08477x$10^{-4}$AB + 1.02942x$10^{-3}$AC + 5.63881x$10^{-4}$BC | 0.98 | <0.0001 | 0.1086 |
| **Overall linking** | Y= 0.050962A + 0.049824B + 0.035139C + 1.36318x$10^{-4}$AB + 8.00833x$10^{-4}$AC + 4.06117x$10^{-4}$BC | 0.98 | <0.0001 | 0.1729 |

A: var. *tenerum* leaf powder; B: *P. amaryllifolius* Roxb leaf powder; C: *C. indicum* L. flower powder.

$R^2$ fitted model ≥ 0.75. The probability of the model was significantly different (p<0.05).



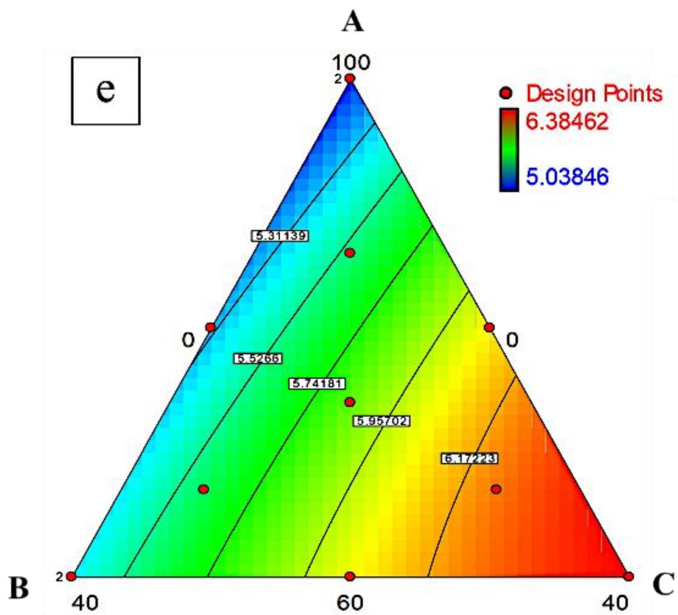



**Figure 5.** Contour plot of optimized formula (a), color (b), taste (c), after taste (d), and overall liking (e) attributes

A: var. *tenerum* leaf powder; B: *P. amaryllifolius* Roxb leaf powder: C: *C. indicum* L. flower powder

**Total polyphenol content and antioxidant activity of var. *tenerum* leave powder tea added with *C. indicum* L. flower powder:** The TPC and antioxidant activity of drinks made from powdered *G. gnemon* var. *tenerum* leaves and *C. indicum* L. flowers (ratio 60:40) were rechecked. Results revealed that the drinks exhibited DPPH at 25.08±0.93 mg GAE/g DW, 11.70±0.26 mg GAE/g DW, 39.10±0.81 mg TE/g DW, and 332.33±6.94 mg AE/g DW, as shown in Table 8. Results indicated that the drink had higher TPC and antioxidant activity compared to without adding *C. indicum* ($T_4$) because *C. indicum* L. flower powder contained higher TPC and antioxidant activity at 32.07±0.15 mg GAE/g DW, 17.76±0.56 mg GAE/g DW, 60.99±1.76 mg TE/g DW, and 516.62±15.08 mg AE/g DW (Table 7). Powder of *C. indicum* L. flower contained several phenolic compounds, including mono-caffeoylquinic acids, di-caffeoylquinic acids, luteolin, apigenin, and anthocyanins [13]. *C. indicum* L. flower powder showed antioxidant activity with $IC_{50}$ values of 1.109-1.350 µg/ml [50]. Moreover, it was reported that *Chrysanthemum* flower extract containing high luteolin could reduce serum uric acid levels in treated humans but did not cause any abnormalities in 12 weeks [51].

*Camellia sinensis*, *Aspalathus linearis*, *Rosmarinus officinalis*, *Diospyros kaki,* and *Sasa borealis,* which are a popular choice for drinking tea to promote health and reduced risk disease as well as meet the experience of taste and flavor to compare with this experiment, results showed that commercial green tea provided higher TPC and antioxidants activity than general leafy teas [52, 53] as shown in Table 9. However, Liang tea mixed gave a higher antioxidant activity than others when used with ascorbic acid equivalent as standard. It confirmed that Liang tea mixed was a good drink compared to others. Therefore, mixed Liang tea can be used as an alternative healthy or functional ingredient.

**Table 8.** Comparison of total polyphenol content and antioxidant activity between *C. indicum* L. flower powder, $T_4$, and the optimized formula.

| Parameter | *C. indicum* L. flower powder | $T_4$ | Optimized formula |
|---|---|---|---|
| Total polyphenol content | 32.07±0.15[a] | 20.38±0.27[c] | 25.08±0.93[b] |
| DPPH (mg GAE /g DW) | 17.76±0.56[a] | 5.74±0.26[c] | 11.70±0.26[b] |
| DPPH (mg TE /g DW) | 60.99±1.76[a] | 19.09±0.83[c] | 39.10±0.81[b] |
| DPPH (mg AE/g DW) | 516.62±15.08[a] | 162.32±7.13[c] | 332.33±6.94[b] |

Mean±SD. Values in a row followed by different superscripts are significantly different (p<0.05). $T_4$: Treatment 4

**Table 9.** Comparison of total polyphenol content and antioxidant activity between Liang tea added *C. indicum* L. flower powder with other teas.

| Teas | Total polyphenol content mg GAE/g DW | DPPH (mg GAE /g DW) | DPPH (mg TE /g DW) | DPPH (mg AE/g DW) | Ref |
|---|---|---|---|---|---|
| **Liang tea mixed** | 25.08±0.93 | 11.70±0.26 | 39.10±0.81 | 332.33±6.94 | This study |
| **Sea Dyke green tea (Commercial brand)** | 113.67±14.75 | n.d. | n.d. | 184.57±17.37 | [52] |
| **Leafy teas** | | | | | [53] |
| **Green tea (*Camellia sinensis*)** | 82.21±1.76 | n.d. | n.d. | 82.54±0.46 | |
| **Rooibos tea (*Aspalathus linearis*)** | 38.66±0.11 | n.d. | n.d. | 9.06±0.35 | |
| **rosemary tea (*Rosmarinus officinalis*)** | 30.84±0.93 | n.d. | n.d. | 15.06±0.57 | |
| **persimmon leaf tea (*Diospyros kaki*)** | 14.72±0.26 | n.d. | n.d. | 12.35±0.22 | |
| **bamboo leaf tea (*Sasa borealis*)** | 11.50±0.82 | n.d. | n.d. | 2.63±0.15 | |

Mean±SD. Values in a row followed by different superscripts are significantly different (p<0.05). n.d. mean not determined.

## CONCLUSIONS

The withering treatment gave a high TPC of *G. gnemon* var. *tenerum* leaf powder, while the DPPH scavenging activity of the steamed-rolling treatment was the highest but recorded the lowest TPC. The pretreatments did not significantly influence sensory scores and cannot improve the unpleasant flavor of *G. gnemon* var. *tenerum* leaf tea. However, sensory improvement of the *G. gnemon* var *tenerum* tea was achieved with added fragrant plants. The optimized formula contained powder of *G. gnemon* var t*enerum* leaves and *C. indicum* flowers at a ratio of 60:40, which is close to F4. The mixed powder of Liang with *C. indicum* flowers tea gave an overall liking score of 6.38±1.63, with acceptance at 78.85%. TPC was 25.08±0.93 mg GAE/g DW, and DPPH scavenging capacity was 11.70±0.26 mg GAE/g DW, 39.10±0.8 mg TE/g DW, and 332.33±6.94 mg AE/g DW. While adding C. indicum did not significantly enhance sensory scores, it notably increased TPC and antioxidant activity compared to tea without *C. indicum* addition.

The mixed Liang tea shows potential as a functional beverage with health-promoting properties. However, sensory score improvement is further needed to investigate for broader utilization. In addition, high-performance liquid chromatography (HPLC) analysis should be done to complete the chemical characterization of the matrix and deepen the main compounds. A multitarget approach should investigate the antioxidant potential.

**Abbreviations:** TPC: total polyphenol content, GAE: gallic acid equivalent, DW: dry weight, DPPH: 2,2-Diphenyl-1-picrylhydrazylradical, TE: Trolox equivalent, AE: ascorbic acid equivalent, CRD: wholly randomized design, RCBD:

randomized complete block design, ANOVA: One-way analysis of variance, DMRT: Duncan's new multiple range test, PPO: polyphenol oxidase enzyme, ABTS: 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, FRAP: ferric reducing antioxidant power, HPLC: high-performance liquid chromatography

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**Author contributions:** Rungnapa Anankarnkit: Conceptualization; investigation; writing – original draft; methodology; validation; writing – formal analysis – review and editing. Suppakarn Muneewan: Conceptualization; investigation; writing – formal analysis; data curation. Worapong Usawakesmanee: Supervision; project administration; writing – review and editing; conceptualization; investigation. Sunisa Siripongvutikorn: Conceptualization; investigation; supervision; writing – review and editing.

**Conflict of interest statement:** There are no conflicts to declare

**Data availability statement**: the data that support the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Anisong N., Siripongvutikorn S., Wichienchot S., Puttarak P. A comprehensive review on nutritional contents and functional properties of *Gnetum gnemon* Linn. *Food Science and Technology*. 2022; 42: e100121. DOI: https://doi.org/10.1590/fst.100121
2. Suksanga A., Siripongvutikorn S., Yupanqui C. T., Leelawattana R. The potential antidiabetic properties of Liang (*Gnetum gnemon* var. *tenerum*) leaves. *Food Science and Technology.* 2022; 42: e64522. DOI: https://doi.org/10.1590/fst.64522
3. Siripongvutikorn S., Usawakesmanee W., Pisuchpen S., Khatcharin N., Rujirapong C. Nutritional content and microbial load of fresh Liang, *Gnetum gnemon* var. *tenerum* leaves*. Foods*. 2023; 12(20):3848. DOI: https://doi.org/10.3390/foods12203848
4. Siripongvutikorn S., Usawakesmanee W., Pisuchpen S., Khatcharin N., Rujirapong C. Quality changes during storage in Thai indigenous leafy vegetable, Liang leaves (*Gnetum gnemon* var. *tenerum*) after different preparation methods. *Italian Journal of Food Science*. 2023; 35(3):1-16. DOI: https://doi.org/10.15586/ijfs.v35i3.2346
5. Suksanga A., Siripongvutikorn S., Leelawattana R., Yupanqui C. T. The antihyperglycemic effect of crude liang (*Gnetum gnemon* var. *tenerum*) leaves powder on *Wistar* rats. *Journal of Nutrition and Metabolism.* 2023; 2023(1): 5630204. DOI: https://doi.org/10.1155/2023/5630204
6. Anisong N., Siripongvutikorn S., Puttarak P., Wichenchot S. Fecal fermentation and gut microbiota modulation of dietary fibre and polyphenols from *Gnetum gnemon* Linn. *leaves. Journal of Bioactive Carbohydrate and Dietary Fibre*. 2023; 30:100380. DOI: https://doi.org/10.1016/j.bcdf.2023.100380
7. Martirosyan D., Lampert T., Lee M. A comprehensive review on the role of food bioactive compounds in functional food science*. Functional Food Science*; 2022.; 3(2):64-78. DOI: https://doi.org/10.31989/ffs.v2i3.906
8. Martirosyan D., Stratton S. Quantum and tempus theories of function food science in practice. *Functional Food Science*; 2023; 3(5):55-62. DOI: https://www.doi.org/10.31989/ffs.v3i5.1122
9. Hou Z., Wang Y., Xu S., Wei Y., Bao G., Dai Q., Deng W., Ning J. Effects of dynamic and static withering technology on volatile and nonvolatile components of Keemun black tea using GC-MS and HPLC combined with chemometrics. *LWT-Food Science and Technology.* 2020; 130:109547. DOI: https://doi.org/10.1016/j.lwt.2020.109547
10. Tsurunaga Y., Kanou M., Ikeura H., Makino M., Oowatari Y., Tsuchiya I. Effect of different tea manufacturing methods on the antioxidant activity, functional components, and aroma compounds of *Ocimum gratissimum*. *LWT- Food Science and Technology*. 2022; 169: 114058. DOI: https://doi.org/10.1016/j.lwt.2022.114058
11. Chen Q., Yu P., Li, Z., Wang Y., Liu Y., Zhu, Y., Fu, H. Re-rolling treatment in the fermentation process improves the aroma quality of black tea*. Foods*. 2023; 12 (19):3720. DOI: https://doi.org/10.3390/foods12193702
12. Wakte K. V., Nadaf A. B., Thengane R. J., Jawali, N. *Pandanus amaryllifolius* Roxb. cultivated as a spice in coastal regions of India. *Genetic Resources and Crop Evolution*; 2009; 56:735-740. DOI: https://doi.org/10.1007/s10722-009-9431-5
13. Yan L., Zhang A., Qin X., Yu H., Ji X., He S., Zong Y., Gu C., Feng Z., Hu L., Lu Z. Changes in key volatile components associated with leaf quality of *Pandanus amaryllifolius* Roxb. alongside growth duration. *Food Chemixtry*: X. 2025; 25:102126. DOI: https://doi.org/10.1016/j.fochx.2024.102126

14. Han A-R., Nam B., Kim B-R., Lee K-C., Song B-S., Kim S. H., et al. Phytochemical composition and antioxidant activities of two different color chrysanthemum flower teas. *Molecules;* 2019; 24(2):329. DOI: https://doi.org/10.3390/molecules24020329
15. Sharma N., Radha., Kumar M., Kumari N., Puri S., Rais N., et al. Phytochemicals, therapeutic benefits and applications of chrysanthemum flower: A review. *Heliyon*; 2023; 9(10):e20232. DOI: https://doi.org/10.1016/j.heliyon.2023.e20232
16. Zhu L., Liao J., Liu Y., Zhou C., Wang X., Hu Z., Huang B., Zhang, J. Integrative metabolome and transcriptome analyses reveal the molecular mechanism underlying variation in floral scent during flower development of *Chrysanthemum indicum* var. aromaticum. *Front Plant Sci*; 2022; 13:919151. DOI: https://doi.org/10.3389/fpls.2022.919151
17. Srisook K., Jinda S., Srisook E. Anti-inflammatory and antioxidant effects of *Pluchea indica* leaf extract in TNF-α-induced human endothelial cells. *Walailak Journal of Science and Technology;* 2021; 18(10):10271. DOI: https://doi.org/10.48048/wjst.2021.10271
18. Latimer G. W., Association of official analytical chemists international. Official Methods of Analysis of AOAC International. 21st ed. *AOAC International*; 2019.
19. Shittu T. A., Lawal M. O. Factors affecting instant properties of powdered cocoa beverages. *Food Chemistry*; 2007; 100(1):91-98. DOI: https://doi.org/10.1016/j.foodchem.2005.09.013
20. Suksanga A., Siripongvutikorn S., Leelawattana R., Yupanqui C. T., Idowu, A. O. Assessment of biological activities, acute and sub-chronic toxicity of Liang (*Gnetum gnemon* var. *tenerum*) leaves powder, a natural product. *Biol. Pharm. Bull;* 2023; 46(12):1666-1675. DOI: https://doi.org/10.1248/bpb.b23-00208
21. Xie Y., Li X., Chen C., Zhang W., Yu X., Xiao H., Lu F. Effects of steam and water blanching on drying characteristics, water distribution, microstructure, and bioactive components of *Gastrodia elata*. *Plants;* 2023; 12(6):1372. DOI: https://doi.org/10.3390/plants12061372
22. Qin W., Yamada R., Araki T., Cai Y., Ogawa Y. Effect of processing step and condition for Japanese green tea manufacturing process on structural attribute of tea leaves and antioxidant activity of tea infusion. *Journal of Food Science and Agricultural Technology;* 2018; 4:41-45.
23. Akyildiz A., Mertoglu T. S., Agcam E. Kinetic study for ascorbic acid degradation, hydroxymethylfurfural and furfural formations in orange juice. *Journal of Food Composition and Analysis;* 2021; 102:103996. DOI: https://doi.org/10.1016/j.jfca.2021.103996
24. Lee S., Choi Y., Jeong H. S., Lee J., Sung J. Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. *Food Sci Biotechnol*; 2018; 27(2):333-342. DOI: https://doi.org/10.1007/s10068-017-0281-1
25. Wickramasinghe Y. W. H., Wichramasinghe I., Wijesekara I. Effect of steam blanching, dehydration temperature & time, on the sensory and nutritional properties of a herbal tea developed from *Moringa oleifera* leaves. *International Journal of Food Science;* 2020(1):5376280. DOI: https://doi.org/10.1155/2020/5376280
26. Okibe F. G., Jubril B. Paul E. D., Shallangwa G. A., Dallatu Y. A. Effect of cooking methods on proximate and mineral composition of fluted pumpkin (*Telfairia occidentalis*) leaves. *International Journal of Biochemistry Research & Review*;2016; 9(2):1-7.
27. Zapata J. E., Sepúlveda C. T., Álvarez A. C. Kinetics of the thermal degradation of phenolic compounds from achiote leaves (*Bixa orellana* L.) and its effect on the antioxidant activity. *Food Science and Technology*; 2022; 42(1): e30920. DOI: https://doi.org/10.1590/fst.30920
28. Lee L., Kim Y., Park J., Kim Y., Kim S. Changes in major polyphenolic compounds of tea (*Camellia sinensis*) leaves during the production of black tea. *Food Science and Biotechnology;* 2016; 25:1523-1527. DOI: https://doi.org/10.1007/s10068-016-0236-y
29. Zhang S., Wu S., Yu Q., Shan X., Chen L., Deng Y., et al. The influence of rolling pressure on the changes in non-volatile compounds and sensory quality of congou black tea: The combination of metabolomics, E-tongue, and chromatic differences analyses. *Food Chemistry;* 2023; 20:100989. DOI: https://doi.org/10.1016/j.fochx.2023.100989
30. Ding J., Mei S., Gao L., Wang Q., Ma H., Chen X. Tea processing steps affect chemical compositions, enzyme activities, and antioxidant and anti-inflammatory activities of coffee leaves. *Food Frontiers;* 2021; 3(3): 505-516. DOI: https://doi.org/10.1002/fft2.136
31. Złotek U., Gawlik-Dziki U., Dziki D., Świeca M., Nowak R., Martinez E. Influence of drying temperature on phenolic acids composition and antioxidant activity of sprouts and leaves of white and red quinoa. *Journal of Chemistry;* 2019; 2019(1):7125169. DOI: https://doi.org/10.1155/2019/7125169
32. Wojdyło A., Figiel A., Lech K., Nowicka P., Oszmiański J. Effect of convective and vacuum–microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. *Food Bioprocess Technol;* 2014; 7:829–841. DOI: https://doi.org/10.1007/s11947-013-1130-8
33. Gui A., Gao S., Zheng P., Feng Z., Liu P., Ye F., et al. Dynamic changes in non-volatile

components during steamed green tea manufacturing based on widely targeted metabolomic analysis. *Foods;* 2023; 12(7):1551. DOI: https://doi.org/10.3390/foods12071551

34. Everette J. D., Bryant Q. M., Green A. M., Abbey Y. A., Wangila G. W., Walker R. B. Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. *J. Agric. Food Chem.* 2010; 58(14):8139-8144. DOI: https://doi.org/10.1021/jf1005935
35. Ministry of public health. Ascorbic acid. Dietary Reference Intake for Thais 2020: vitamin C. Ministry of Public Health; 2020.
36. Hwang S., Lee J. Comparison of antioxidant activities expressed as equivalents of standard antioxidant. *Food Science and Technology* 2023; 43:e121522. DOI: https://doi.org/10.1590/fst.121522
37. Owolabi I. O., Yupanqui C. T., Siripongvutikorn S. Enhancing secondary metabolites (emphasis on phenolics and antioxidants) in plants through elicitation and metabolomics. *Pakistan Journal of Nutrition* 2018; 17(9):411-420.
38. Saetan P., Usawakesmanee W., Siripongvutikorn, S. Influence of hot water blanching process on phenolic profile and antioxidant activity of Cinnamomum porrectum herbal tea. *Functional Foods in Health and Disease;* 2016; 6(12):836-854. DOI: https://doi.org/10.31989/ffhd.v6i12.315
39. Yamauchi M., Kitamur, Y., Nagano H., Kawatsu J., Gotoh H. DPPH Measurements and Structure—Activity Relationship Studies on the Antioxidant Capacity of Phenols. *Antioxidants* 2024; 13(3):309. DOI: https://doi.org/10.3390/antiox13030309
40. Guo Q., Yu J., Zhao Y., Liu T., Su M., Jia Z., et al. Identification of fishy odor causing compounds produced by *Ochromonas* sp. and *Cryptomonas ovate* with gas chromatography-olfactometry and comprehensive two-dimensional gas chromatography. *Science of The Total Environment* 2019; 671:149-156. DOI: https://doi.org/10.1016/j.scitotenv.2019.03.370
41. Shinfuku Y., Takanashi H., Nakajima T., Kasuga I., Akiba M. The status quo of causal substance exploration for fishy odor in raw water for taps. *Journal of Water and Environment Technology* 2022; 20(2):29-44. DOI: https://doi.org/10.2965/jwet.21-135
42. Laska M. Olfactory perception of 6 amino acids by human subjects. *Chemical Senses* 2010; 35(4):279-287. DOI: https://doi.org/10.1093/chemse/bjq017
43. Plaza N. C., García-Galbis M. R., Martínez-Espinosa R. M. Effects of the usage of l-cysteine (l-cys) on human health. *Molecules* 2018; 23(3):575. DOI: https://doi.org/10.3390/molecules23030575
44. Martínez Y., Li X., Liu G., Bin P., Yan W., Más D., et al. The role of methionine on metabolism, oxidative stress, and diseases. *Amino Acids* 2017; 49:2091-2098. DOI: https://doi.org/10.1007/s00726-017-2494-2
45. Wu G., Bazer F. W., Burghardt R. C., Johnson G. A., Kim S. W., Knabe D. A. et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids* 2010; 40(4):1053-1063. DOI: https://doi.org/10.1007/s00726-010-0715-z
46. Sarstedt M., Mooi E. A concise guide to market research: regression analysis. Springer, Berlin, Heidelberg; 2014. DOI: https://doi.org/10.1007/978-3-642-53965-7_7
47. Pimenta C. D., Silva M. B., Campos R. L. de M., Junior W. R. de C. Desirability and design of experiments applied to the optimization of the reduction of decarburization of the process heat treatment for steel wire sae 51B35. *American Journal of Theoretical and Applied Statistics* 2018; 7(1):35-44. DOI: https://doi.org/10.11648/j.ajtas.20180701.15
48. Liu G., Duan H., Zheng Y., Guo J., Wang D., Yan W. Differences in the determination of volatile organic compounds between *Chrysanthemum morifolium* ramat. and *Chrysanthemum indicum* L. (Wild Chrysanthemum) by HS-GC-IMS. Molecules. 2024; 29(19):4609. DOI: https://doi.org/10.3390/molecules29194609
49. Choi HS., Kim, GH. Volatile flavor composition of gamguk (*Chrysanthemum indicum*) flower essential oils. *Food Sci Biotechnol* 2011; 20:319–325. DOI: https://doi.org/10.1007/s10068-011-0045-2
50. Dolongtelide J. I., Fatimawali F., Tallei T. E., Suoth E. J., Simbala H. E. I., Simbala I., et al. In vitro antioxidant activity of *Chrysanthemum indicum* flowers extract and its fractiont. *Malacca Pharmaceutics* 2023; 1(2):43-47. DOI: https://doi.org/10.60084/mp.v1i2.26
51. Takara T., Yamamoto K., Suzuki N., Yamashita S., Lio S., Kakinuma T. et al. Effects of luteolin-rich *chrysanthemum* flower extract on purine base absorption and blood uric acid in Japanese subjects. *Functional Foods in Health and Disease* 2022; 12(1):12-25. DOI: https://doi.org/10.31989/ffhd.v12i1.863
52. Chan E. W. C., Lim Y. Y., Chew Y. L. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. *Food Chemistry* 2007; 102: 1214-1222. DOI: https://doi.org/10.1016/j.foodchem.2006.07.009
53. Oh J., Jo H., Cho A. R., Kim S., Han J. Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Control* 2013; 31:403-409. DOI: https://doi.org/10.1016/j.foodcont.2012.10.021