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# Effect of different drying methods on the antioxidant properties of *Vitex negundo* Linn. tea

*Rabeta, M. S. and Vithyia, M.

*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*

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**Abstract**

This study was done to determine the effects of different thermal drying methods (sun drying, microwave drying and hot air oven drying) on the total phenolic content (TPC), total anthocyanin content and the antioxidant properties of *Vitex negundo* (VN) tea. Significant decline ($P < 0.05$) in antioxidant properties of hot air oven drying shows that this method is not the best method to preserve antioxidant compounds in VN tea. As a conclusion, microwave drying has been found to be a good method for maintain the TPC, anthocyanin content and AEAC in dried sample of VN tea.



## Introduction

Herbs are valued for its specific aroma, taste, putative physiological effect and medicinal properties which appeal to sense of taste, smell, and sight and therefore promote continuous development of functional foods and drinks (Yokozawa *et al.*, 1998; Tsai *et al*., 2007). Such herbal remedies often consumed in the form of tea, where boiling water are added to steep infusion of dried plant parts.

Tea has been used as a daily beverage and crude medicine in China for thousands of years (Yen and Chen, 1995). Asian countries, Africa and South America produce different types of teas (Hicks, 2001). This aromatic beverage prepared from cured leaves is the second most popular beverage in the world after water (Cabrera *et al*., 2003). In Asian countries, tea drinking is a ritual and life style, however in European countries tea consumption is occasional and usually European choose a wide variety of fruit teas or traditional herbal infusions. Till date, tea consumption depends primarily on the type and mode of preparations (Horzic *et al.*, 2009).

Major constituents of tea component vary with species, season, climate, leaf maturity, cultivation conditions and horticultural practices (Polovka *et al*., 2003; Chan *et al*., 2007). Old tea leaves are not used in tea processing and often considered as agricultural waste (Farhoosh *et al.*, 2007). Herbal tea is a polyherbal formulation of different medicinal plants that is a rich source of antioxidant. However, tea manufacturing processes can greatly affect the oxidation of tea polyphenols. Food antioxidants often lost as a result of sterilisation, pasteurisation, dehydration and prolonged storage (Manzocco *et al*., 1998).

*Vitex negundo* (VN) is a medicinal plant which is a source of natural compounds and has an extensive use in the treatment of various diseases (Vinuchakkaravarthy *et al*., 2011), known as Lemuni in Malays community. This herbs is utilized to treat female disorders especially disorders that are linked with female reproductive system and to decrease sexual desire. Moreover, it is also use as antipyretic, anti-inflammatory, anti-androgenic and for analgesic activities (Vimal *et al*., 2011).

Antioxidant activity in food products can also be affected by various agricultural cultivation practices, assortment, industrial handing, packaging, and storage measures. Storage environment is vital to preserve antioxidant capacity of herbal tea (Naithani *et al*., 2006). However, processing and storage does not entirely responsible for depletion of antioxidant properties in foods. In some cases, these factors can also induce formation of compounds with novel antioxidant that maintain or even enhance the overall antioxidant potential of food (Manzocco *et al*., 1998). Dietary supplements of antioxidants are well accepted by many as it is proven to improve defense mechanism in human body (Fu *et al.*, 2011).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl

*Corresponding author.
Email: *rabeta75@yahoo.com*

toluene (BHT) are widely used to neutralise free radicals and function as inhibitors in lipid peroxidation. It also enables stabilizing property in fat containing foods (Zargar *et al*., 2011). Lipid oxidation in food often leads to significant loss of nutritional quality and causes formation of toxic compounds. In addition, synthetic antioxidants such as BHA, BHT and tert-Butyhydroquinone (TBHQ) are commonly used in the food industry as prospective inhibitors (Chaieb *et al*., 2011).

Extensive uses of BHA and BHT have been related with toxicity and carcinogenicity effects. Therefore, the usage of synthetic antioxidants is often doubted. Currently, researchers have tried to find replacement for synthetic antioxidants from natural antioxidants such as flavonoids, tocopherols and ascorbic acid. Medicinal plants exhibit potential antioxidant activity due to presence of various phytochemicals and have always been regarded as source of natural bioactive compounds (Zargar *et al*., 2011).

Many studies had shown that phenolic compounds such as anthocyanins exhibit strong antioxidant activity in plants (Özgen *et al*., 2010). The objectives of this study were to determine the total phenolic content (TPC) in VN tea using Folin-Ciocalteu colorimetric method, determine 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging activity and total anthocyanins content in VN tea and identify the effects of different drying methods on TPC and Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) in VN tea. Previous study had showed that the fresh leaves of VN contained higher amount of total phenolics compounds compared to the flowers (Rabeta and An Nabil, 2013), to our knowledge, the effects of different thermal drying treatments are poorly studied.

## Materials and Methods

*Plant material*

*Vitex negundo* leaves were harvested by hand in Kuala Kurau, Perak. The sample was identified by Mr. Adnan Jaafar and was deposited in the Herbarium Unit of School of Biological Sciences, Universiti Sains Malaysia (USM Herbarium 11461). Only young, mature and good shape leaves were used for the processing of tea. All the other part of the plant such as flower, stem and root were discarded.

*Tea processing*

The preliminary step was to produce VN tea. The tea preparation consisted of four steps, namely withering, rolling, fermentation, drying and storage. The processing of VN tea took approximately 3 weeks. This method of tea processing was adapted and modified from *Camellia theifera* black tea preparation described by (Adisewojo, 1982).

*Thermal drying methods*

The VN tea leaves were subjected to three different thermal drying methods namely sun drying, microwave drying and hot air oven drying. The tea leaves were spread evenly and approximately one gram of tea leaves was used. Drying treatments were done triplicate. All tea leaves used for drying were from the same batch.

*Sample preparation*

Tea extracts were prepared using hot water extraction method described previously by Chan *et al.* (2007) with slight modification. VN tea leaves were ground with a mortar and pestle into powder. Approximately 1 g of of tea in powder form was extracted with 50 ml of boiling water with continuous swirling at room temperature (20$^{o}$C ± 5$^{o}$C) in incubator shaker (SI-600R, Korea) at 120 rpm. Infusion was allowed to steep for 1 hour after centrifuged in ultracentrifuge (Thermo Scientific, US) for 15 minutes at 2300 x g. The extract was filtered using filter paper (Whatman No 4) and stored in a dark container at -4$^{o}$C for further analysis. Filtrate was prepared in triplicate in test tubes without further treatment.

*Determination of total phenolic content (TPC)*

Total phenolic content in VN tea was determined using the Folin–Ciocalteu assay according to the method described previously by Chan *et al.* (2007). Three hundred microliters of tea extract samples was introduced into the test tubes. Then, 1.5 mL of Folin–Ciocalteu's reagent which was diluted ten times was added, followed by 1.2 mL of sodium carbonate (7.5% w/v). Subsequently, test tubes were left to stand for 30 min at room temperature before proceeding to measure absorbance using UV-visible spectrophotometer (UV Mini-1240, Shimadzu, Japan) at 765 nm. Calibration curve was prepared using aqueous gallic acid with 0.02–0.1 mg / mL, where $r^2$ = 0.9998. The results were expressed in terms of mg GAE/100 g material.

*Determination of total anthocyanins content*

Total anthocyanins content of VN tea was determined by using pH differential method as described in Association of Analytical Chemists (AOAC) Official (Method 2005.02). Firstly, pH 1.0 buffer solution 0.025M potassium chloride was prepared. 1.86 g KCl was weighted and added into

a beaker. Approximately 980 mL of distilled water was added. The pH was measured and adjusted to pH 1.0 (±0.05) with 6.3 mL of HCl. The solution was then transferred to a 1 L volumetric flask and diluted to volume with distilled water. Next, pH 4.5 buffer solution 0.4 M sodium acetate was prepared.

The appropriate dilution factor was determined by diluting the extract portion with pH 1.0 buffer using UV-visible spectrophotometer (UV Mini-1240, Shimadzu, Japan) until the absorbance at 520 nm is within the linear range of the spectrophotometer. Using this dilution factor, 2 dilutions of the test sample was prepared, where one with pH 1.0 buffer and the other with pH 4.5 buffer. Absorbance of the test portion diluted with pH 1.0 buffer and pH 4.5 buffers was determined at 520 nm and 700 nm.

*Determination of 1, 1-diphenyl-2-picrylhydrazyl DPPH free-radical scavenging activity*

The ability of compounds to act as free radical scavengers or hydrogen donor and antioxidant activity can be assessed using the DPPH assay. DPPH radical-scavenging capacity in VN tea was determine according to the method described by Chan *et al.* (2009). About 1 mL of extract with different dilution was added to 2 mL of 1, 1-diphenyl-2-picrylhydrazyl (5.9 mg per 100 mL 100% methanol). Absorbance was measured using UV-visible spectrophotometer (UV Mini-1240, Shimadzu, Japan) at 517 nm upon standing for 30 min at room temperature. Radical Scavenging activity is expressed as AEAC in mg ascorbic acid/100 g and was calculated as:

$$\text{AEAC (mg AA/100 g)} = \frac{IC_{50\ (\text{ascorbate})}}{IC_{50\ (\text{sample})}} \times 10^5$$

where $IC_{50}$ of ascorbic acid used to calculate AEAC was 0.00387 mg/ml.

*Statistical analysis*

All of the results obtained were as means ± SD. Analysis of variance (ANOVA) was used to determine the significant differences for multiple comparisons which was completed using the Tukey Honestly Significant Different (HSD) test at $\alpha = 0.05$. All of these were carried out using SPSS statistical package (ver.16.0).

## Results and Discussion

*Sun drying*

The TPC and Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) of VN that are subjected to sun drying on 3 consecutive days was presented in Table 1. All the results were reported based on fresh sample of tea after processing. Average temperature of the 3 consecutive days was 34.5 ± 3.0˚C.

Table 1. TPC and AEAC of *Vitex Negundo* subjected to sun drying on 3 consecutive days

| | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| TPC | 52.5± 3.1[a] | 54.7± 8.3[a] | 51.9± 3.7[a] |
| AEAC | 58.8 ± 7.8[a] | 59.5 ± 9.2[a] | 57.5± 5.5[a] |

Results are presented as means ± standard deviation (n = 3). For each row within the same species, values followed by the same in superscript are not statistically different at P > 0.05 as measured by the Tukey HSD test. TPC and AEAC, expressed in mg GAE/100 g and mg AA/100 g respectively.

On the first day, the TPC reported was 52.5 ± 3.1 mg GAE/100 g. The TPC reported on second day was slightly higher at 54.7 ± 8.3 mg GAE/100 g. On the third day, the TPC value was reported as 51.9 ± 3.7 mg GAE/100 g. On the other hand, the AEAC after sun drying of VN tea was reported to be 58.8 ± 7.8 mg AA/100 g on the first day. Similar to TPC on the second day, AEAC was reported to be 59.5 ± 9.2 mg AA/100 g. On the third day, AEAC was 57.5 ± 5.5 mg AA/100 g.

Based on the result reported, sun drying resulted the loss of the TPC and AEAC insignificantly ($p > 0.05$). The reduction of TPC value after sun drying may be caused by the enzymatic reaction during the process (Lim and Murtijaya, 2007). Moreover, it also causes enzymes degradation and loss of antioxidant enzyme activities. Declines in antioxidant properties are often results of loss of other bioactive properties (Chan *et al.*, 2009).

*Microwave drying*

All the results were reported based on fresh sample of tea after processing. The TPC of control was reported as 1365 ± 50 mg GAE/100 g. When the leaves were subjected to drying for 2 minutes, TPC reported was 1348 ± 67 mg GAE/100 g. After 4 minutes of drying, TPC was 1355 ± 48 mg GAE/100 g which was almost similar. When VN was dried for 8 minutes, TPC reported was 1369 ± 62 mg GAE/100 g. AEAC of control sample was reported to be 1323 ± 65 mg AA/100 g. When the tea leaves were subjected to microwave drying for 2 minutes, AEAC was 1446 ± 82 mg AA/100 g. AEAC was reported to be 1454 ± 50 mg AA/100 g after drying in microwave oven for 4 minutes. Lastly upon drying for 8 minutes, AEAC reported was 1395 ± 105 mg AA/100 g. Based on the result reported, it is proven that microwave drying does not cause any significant changes ($p > 0.05$) in TPC and AEAC. Furthermore, increase in drying time does not affect the AOP of VN tea. Table 2 showed the TPC and AEAC of VN tea subjected to 2 min, 4

Table 2. TPC and AEAC of *Vitex negundo* tea subjected to 2 min, 4 min and 8 min of microwave drying

| | Control | 2 min | 4 min | 8 min |
|---|---|---|---|---|
| TPC | 1365± 50[a] | 1348 ± 67[a] | 1355 ± 48[a] | 1369± 62[a] |
| AEAC | 1323± 65[a] | 1446 ± 82[a] | 1454± 50[a] | 1395± 105[a] |

Results are based on fresh sample of tea leaves and presented as mean ± standard deviation (n = 3). Values followed by the same letter in superscript are not statistically different at P > 0.05 as measured by Tukey HSD test. TPC and AEAC are expressed in mg GAE/100 g and mg AA/100 g respectively.

Table 3. TPC and AEAC of *Vitex negundo* tea subjected to 45°C, 75°C and 95°C of Hot Air Oven Drying

| | | Hot Air Oven | | |
|---|---|---|---|---|
| | Control | 45°C | 75°C | 95°C |
| TPC | 1584 ± 56[a] | 1499± 80[a] | 785± 33[b] | 752 ± 88[b] |
| AEAC | 1778 ± 23[a] | 1008 ± 78[a] | 988 ± 33[b] | 959± 10[b] |

TPC and AEAC expressed in mg GAE/100 g and mg AA/100 g respectively. Results are based on fresh sample of tea leaves and presented as mean ± standard deviation (n = 3). Values followed by the same letter in superscript are statistically different at P < 0.05 as measured by Tukey HSD test. TPC and AEAC are expressed in mg GAE/100 g and mg AA/100 g, respectively.

min and 8 min of microwave drying.

Previous study by Chong and Lim (2012) in VN states that microwave drying does not causes any significant increase (p > 0.05) in the TPC of the leaves. Moreover, microwave dried VN leaves has the advantage of short drying time and low water activity.

*Hot air oven drying*

In hot air oven drying, VN tea was dried at three different temperatures for 30 min with an average temperature of 45°C, between 75°C to 95°C (Table 3). The TPC of control for all three temperatures was reported as 1584 ± 56 mg GAE/100 g. Leaves subjected to drying at 45°C have TPC of 1499 ± 80 mg GAE/100 g. At drying temperature of 75°C, TPC was 785 ± 33 mg GAE/100 g. The TPC content at 95°C was reported as 752 ± 88 mg GAE/100 g which was similar to the result of the leaves dried at 75°C.

AEAC of control sample was reported to be 1778 ± 23 mg AA/100 g. When the tea leaves were subjected to hot air oven drying at 45°C, AEAC was 1008 ± 78 mg AA/100 g. AEAC reported was found to be low at 75°C which was 988 ± 33 mg AA/100 g after drying for 30 minutes. After drying at 95°C, AEAC reported was 959 ± 10 mg AA/100 g. Table 3 showed the TPC and AEAC of VN tea subjected to hot air oven drying at various temperatures.

Based on the result reported, it was clearly shown that hot air oven drying significantly decrease of TPC and AEAC content when the temperature was increased from 45°C to 95°C (p < 0.05). Similar result reported by Chong and Lim (2012) where VN leaves in hot air oven under higher temperatures (70 and 100°C) significantly reduced the TPC and AEAC

Table 4. Total anthocyanin contact of control and dried sample subjected of three different drying treatment

| | Total anthocyanin contact | |
|---|---|---|
| Treatment | Control | Dried sample |
| Sun Drying | 3.15±0.33[b] | 2.10±0.10[b] |
| Microwave Drying | 7.02±0.16[a] | 6.88±0.72[a] |
| Hot Air Oven Drying | 5.11±0.13[a] | 4.72±0.21[b] |

Results are presented as means ± standard deviation (n = 3). For each row within the same species, values followed by the difference in superscript are statistically different at P < 0.05 as measured by the Tukey HSD test. Total anthocyanin content was expressed in mg/kg cyaniding-3-glucoside.

values. Kongsoontornkijkul *et al*. (2006) reported that hot air drying affect the quality of the gooseberry tea drink that has been made from dried gooseberry.

Significant decline (P < 0.05) in antioxidant properties of hot air oven drying shows that this method is not the best method to preserve AOP in tea. The drop of TPC and AEAC in VN leaves from 45 to 75°C due to initial enzymatic degradation of antioxidant compounds where the slow heat transfer in oven drying resulted inefficient denaturation of the enzyme involved (Chong and Lim, 2012). Many studies have reported losses in AOP of plant samples due to oven drying. These losses were mainly observed in vegetables and fruits. This drying treatment applied onto a given plant sample have variable effects on antioxidant properties (AOP). The value of TPC and AEAC continue dropped insignificantly from 75 to 95°C (p > 0.05).

*Total anthocyanins content in different drying methods*

Total anthocyanins in VN tea leaves were expressed as mg cyanidin-3-glucoside/kg. From the result, table 4 showed that in sun drying method, total anthocyanins content reported was 2.10 ± 0.10 mg/kg cyanidin-3-glucoside versus control of 3.15 ± 0.33 mg/kg cyanidin-3-glucoside. Total anthocyanins content in microwave oven drying was reported highest compared others as 6.88 ± 0.72 mg/kg cyanidin-3-glucoside versus control sample of 7.02 ± 0.16 mg/kg cyanidin-3-glucoside. In hot air drying, total anthocyanins content reported was 4.72 ± 0.21 mg/kg cyanidin-3-glucoside versus control sample of 5.11 ± 0.13 mg/kg cyanidin-3-glucoside.

The result clearly showed that anthocyanins content in microwave drying was the highest compared to other drying treatment such as hot air oven drying and sun drying. Maeda-Yamamoto *et al*. (2012) found in their study consuming tea containing anthocyanins would contribute to health maintenance. Therefore, from the data it can be concluded that food industry should opt for microwave drying for as a feasible method of drying as the natural anthocyanins in tea relatively low. Correct method of drying can prevent loss of anthocyanins in tea.

The drying methods were known to have variable

effects on the antioxidant properties of plant samples. Variable in different drying effects include little or no change, significant declines or enhancement in antioxidant properties. Drying process caused changes in chemical composition. In conclusion, microwave drying resulted in highest total anthocyanins and maintaining the antioxidant properties of VN tea.

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