Effect of Hot-Air Drying and Vacuum Drying on Oxalate Contents of *Limnophila Aromatica* and *Limnophila Geoffrayi*

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Abstract

This study investigated the total, soluble and insoluble oxalate contents of two rice paddy herbs (*Limnophila aromatica and Limnophila geoffrayi*) as affected by hot-air and vacuum drying methods at different temperatures (50, 60, 70, and 80°C). Overall, the lowest content of soluble oxalate was observed which a high temperature (70 and 80°C) of hot-air dried samples (3.47 and 3.14 mg/g DW in *L. geoffrayi* and 11.39 and 11.75 mg/g DW in *L. aromatic*, respectively), while soluble oxalate content was highest in the high temperature (80°C) vacuum dried samples (5.84 mg/g DW in *L. geoffrayi* and 18.23 mg/g DW in *L. aromatica*, respectively). With an increase in drying temperature, there was significantly decreased soluble oxalate content in the hot-air dried samples, while oxalate content increased in the vacuum dried samples. Total oxalate contents had slight changes with the same trend for soluble oxalates. On the other hand, insoluble oxalate content showed a contrasting effect with the soluble and total oxalate content. In addition, the results showed that the soluble and total oxalate contents for various drying temperatures for the *L. geoffrayi* species were significantly lower than for the *L. aromatica* species. Hot-air drying at 70°C provided the optimal results with respect to the content of soluble oxalates.

Keywords: Hot-air drying, Vacuum drying, Rice paddy herb, Soluble oxalate

1. Introduction

Several species of *Limnophila* are found in Southeast Asia where it has been used as a spice and a medicinal plant. Two species of *Limnophila* are commonly found in Thailand, *L. aromatica* and *L. geoffrayi*, known as “rice paddy herb” or “Phak Kha Yaeng” (in Thai), and have been used for their flavoring and aroma properties. They are easily cultivated in flooded rice fields and soggy land. The plant is eaten both raw and steamed.

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It is used in Thai cuisine to add flavor. In northeastern Thailand especially, *Limnophila* is among the popular vegetative ingredients used in traditional curry. *L. aromatica*, the cultivated plant, has been shown to have negligible toxicity and to possess diuretic, muscle relaxant and antispasmodic activities. It has been used to treat kidney stones, painful cramps, wounds and ulcers (Do, 1999). *L. geoffrayi* Bonati, the native plant, grows naturally and is used as an antidote for poisons as well as being used as a vegetable in northeastern Thailand. This species is also used as a traditional medicine due to its antipyretic, expectorant, and galactagogue properties (Suksamrarn *et al*., 2003).

These vegetables can be used fresh or dried as a spice. However, fresh vegetables have a very short post-harvest life, causing depreciation in marketplace price. A number of efficient processes have been proposed to extend the shelf life, and to add value to the product as a dried spice. The quality of the dried product is strongly dependent on the drying conditions. In the drying process, heat is required to evaporate moisture from the material and air flow to carry away the evaporated moisture, making drying a high energy consuming operation (Proctor, 1994). There are different heat sources available for drying and these are well discussed in many articles (Jangam and Mujumdar, 2010). Traditionally, the drying process has involved solar drying, however this method is time consuming and weather dependent. Hot-air (HA) drying is the most typically employed commercial method for drying vegetables and fruits (Praveen Kumar *et al*., 2005; Wanyo *et al.,* 2011). Previous research has reported that vacuum-dried products are characterized by their better quality retention, compared to air drying at atmospheric pressure. Vacuum drying has been found to be more effective in drying materials with a higher amount of free moisture (Jaya and Das, 2003; Mitra *et al.*, 2011; Reis *et al*., 2012; Sahari et al., 2008). Despite these research reports, little attention has been given to rice paddy herbs.

One of the limiting factors in the use of rice paddy herbs is the presence of oxalates, which impart a slightly bitter taste or cause irritation when eaten. Oxalates are widely dispersed in plant foods in an easily water-soluble form as potassium, sodium and ammonium oxalates and as insoluble calcium oxalates (Holloway *et al.*, 1989). Oxalate forms robust chelates with dietary calcium, consequently rendering the complex unavailable for absorption and assimilation. It precipitates as insoluble salts accumulating within the renal glomeruli and contributes to the development of renal disease. While different factors have to be considered in the development of renal disease, it is being recommended to restrict the consumption of oxalate-rich foods, particularly for individuals at risk of kidney stone formation (Noonan and Savage, 1999). A diet high in soluble oxalates is well known to be related to an accelerated risk of developing kidney stones, being composed in particular of crystals of calcium oxalate

(Simpson *et al*., 2009). Vegetables are typically consumed as sources of vitamins, minerals, antioxidants and dietary fiber. Some plant ingredients are widely known to contain oxalates (Savage *et al*., 2000), which can be recognized as inhibitors of mineral bioavailability. Several attempts have been made to reduce oxalate content in food materials. Although it has been reported that different drying methods produced taro products with reduced oxalate (Afoakwa *et al*., 2003), they did not completely eliminate it, as itching is still reported by many consumers (Budaraga, 2017). Available reports about the effects of drying on oxalates appear conflicting and inconclusive. Consequently, the aim of the present study was to investigate the influence of two drying methods, namely vacuum and HA drying at different temperatures (50, 60, 70 and 80°C) on oxalate content, including total, soluble and insoluble oxalate contents of two rice paddy herbs (*L. aromatica* and *L. geoffrayi*). We hope to provide useful information for the drying of vegetables or plant foods to reduce the risk of consuming plant foods high in oxalates.

2. Materials and Methods

2.1 Sample preparation

Rice paddy herb samples include two species: *Limnophila aromatica* (Lamarck) Merrill and *Limnophila geoffrayi* Bonati. *L.aromatica* samples were bought from three representative markets in Kalasin province, Thailand, in March 2017. At each market, 10 kg samples were taken from three representative outlets. Single composite samples for each representative market, were prepared by combining about 3 kg of homogenized single sample from three representative outlets and then homogenizing again to obtain a uniform single composite sample. *L. geoffrayi* samples were obtained from local farmers markets in Kalasin, Maha Sarakham and Roi-Et provinces in the northeastern region of Thailand, in March 2017. At each market, 8–10 kg samples were sampled from representative outlets. Single composite samples for each representative market, were prepared by combining about 3 kg of homogenized single sample from three representative outlets and then homogenizing again to obtain a uniform single composite sample. After purchasing, the samples were transported as soon as possible to Food Science and Technology laboratory at the Kalasin University.

Rice paddy herb samples were cleaned and the edible parts were separated. The raw samples were washed with deionized water for 5 min. The ratio between sample and water was 1:4 (w/v). The samples were then allowed to drain at room temperature for 30 min. Afterwards, the samples were divided into nine groups (100 g per group). The first was fresh samples used for control, the second to the fifth were hot-air dried (50–80°C), and the sixth to

the ninth were vacuum dried (50–80°C). All analytical results were expressed on a dry matter basis and experiments performed in triplicate.

2.2 Drying Processes

Samples were subject to two different drying methods, i.e. HA and VC. For each drying method, 100 g of fresh sample was used (in triplicate). In HA drying, the sample was dried by a hot-air drying machine at 50, 60, 70 and 80°C using a hot-air oven (Thermotec 2000 oven, Conterm, New Zealand). For VC drying, the sample was dried in the vacuum dryer at 50, 60, 70 and 80 °C using a vacuum oven (VD23, Binder, Germany). Drying time was set to achieve dried samples containing 7% moisture content. After drying, these samples were allowed to cool to ambient temperature before extraction.

2.3 Oxalate extraction

The total and oxalate contents in test samples were extracted using a modification of a procedure described by Savage et al. (2000). One gram of each finely ground dried sample was extracted with 50 mL HCl (2M) in a water baht at 80°C for 15 min to extract total oxalates. Soluble oxalates were extracted using 50 mL nanopure water at 80°C for 15 min. The extracts were allowed to cool and then transferred quantitatively into 100 mL volumetric flasks and made up to volume. The extract was then centrifuged at 3000 rpm/min for 15 min. The supernatant was filtered through a 0.45 µm filter before injection (20 µL) into the HPLC aperture. The insoluble oxalate content was calculated by the difference between the total and the soluble oxalate content. Samples were analyzed in triplicate.

2.4 Oxalate determination

Analysis of total and soluble oxalates was determined using the methods of Savage *et al*. (2000) where a 15 cm × 4.6mm, 5µm Ascentis RP-Amide column (Supelco Analytical, PA, USA) attached to a cation h+ guard column (Bio-Rad, Richmond, California, USA), using an isocratic elution at 0.5 mL/min with 0.0125 M sulphuric acid (Baker Chemicals, Phillipsburg, NJ, USA) as a mobile phase. Operating conditions were as follows: column temperature, 25°C, injection volume, 20 µL and UV-Diode Array detection at 210 nm. The oxalate peak was identified by comparison of the retention time with that of oxalic acid (99.99%, Sigma, St. Louis, MO, USA). Insoluble oxalate was calculated as the difference between the total oxalate and soluble oxalate (Holloway, 1989). Two standard curves were prepared in the range 1–50 mg/100 mL as all samples came within this linear range; one set of standard solutions was prepared using 0.2 M HCl. This set of standards was used to quantify the total oxalate content of the samples. A further set of standard solutions was prepared by diluting the standard with nanopure water. This set was used to analyze the soluble oxalic acid content of the water extracts.

2.5 Statistical analyses

Analysis of variance (ANOVA) was performed in a completely randomized design, using Duncan's Multiple Range Test. All determinations were done at least in triplicate. The confidence limits used in this study were based on 95% (p<0.05).

3. Results and Discussion

Oxalate assessment of *L. aromatica* and *L. geoffrayi* made from the different air-drying temperatures (50, 60, 70 and 80°C) included soluble, insoluble and total oxalate content. Moisture content was fixed at a 7% dry basis, according to the Thai industrial standards for dried herbs. The average initial moisture content from fresh samples of *L. aromatica* and *L. geoffrayi* were 88.83–91.07% and 84.83–86.61%, respectively. The time required to achieve the above levels of moisture content were 8, 4, 3, and 2 h for HA and 24, 10, 5, and 4 h for hot-air drying under vacuum conditions at the respective temperature (Data not shown).

3.1 Total oxalate content

The values of total oxalate in fresh *L. aromatica* and *L. geoffrayi*, were not significantly different (56.70 mg/g dry basis and 49.22 mg/g dry basis respectively) (data not shown). Table 1 and 2 shows the influence of drying temperature on oxalate contents of *L. aromatica* and *L. geoffrayi*. The results show that *L. aromatica* had a higher value of total oxalate (33.05–40.92 mg/g DW) compared to *L. geoffrayi* (23.03–32.82 mg/g DW). HA drying temperature results in a nonsignificant reduction in the total oxalate content in dried *L. aromatica* and *L. geoffrayi*. Total oxalate content in HA dried *L. aromatica* ranged from 33.05 to 36.68 mg/g DW, *L. geoffrayi* ranged from 23.03 to 29.17 mg/g DW (Table 1 and 2). In the case of vacuum drying of *L. aromatica*, total oxalate content increased nonsignificant when the drying temperature increased (34.16 to 41.61 mg/g DW). For the drying of *L. geoffrayi*, total oxalate content show slightly increased when the drying temperature increased (28.14 to 33.90 mg/g DW).

3.2 Soluble oxalate content

Soluble oxalates, however, may be of interest for kidney stone patients who are trying to decrease their urinary oxalate excretion by avoiding the consumption of oxalate-rich foods (Massey, 2007; Noonan and Savage, 1999; Perera *et al*., 1990; Savage *et al*., 2000). The amount of soluble oxalate in fresh *L. aromatica* (8.95 mg/g dry basis) was significantly higher than that in *L. geoffrayi* (4.03 mg/g dry basis) (data not shown). Soluble oxalate content of *L. aromatica* and *L. geoffrayi* was affected by different drying temperature are presented in Table 1 and 2. The results show that *L. aromatica* had the higher values of soluble oxalate (11.39–18.23 mg/g DW) compared to *L. geoffrayi* (3.14–9.23 mg/g DW). The drying methods used for drying of *L. aromatica* and *L. geoffrayi* showed significantly different soluble oxalates

(p<0.05). Drying temperature is an important factor in drying. Increasing the drying temperature results in an increase of effective moisture diffusivity and a decrease in drying time. However, the quality of the dried product is strongly dependent on the drying process and processing conditions (Pan *et al*., 1999). In addition, the most usual methods for drying, like oven drying and vacuum drying, led to conflicting results (Scholz, 1984). In the case of HA drying, soluble oxalate content decreased when the drying temperature increased from 50 to 80°C. In contrast, soluble oxalate content of vacuum dried samples increased when the drying temperature increased from 50 to 80°C (Table 1 and 2). Overall, the *L. aromatica* dried with HA had a lower content of soluble oxalates, compared with those dried by vacuum drying, while the *L. geoffrayi* dried with two different methods had similar oxalate contents. The results indicate that a high temperature (70 and 80°C) of hot-air dried samples provided the lowest soluble oxalate content compared to the other drying conditions for all the samples studied. On the other hand, vacuum dried samples at high temperatures have shown that the highest content of soluble oxalate. High temperatures are known to cause the calcium oxalate containing cells to collapse, leading to the breakdown of the oxalate structure (Savage *et al*., 2000). However, conflicting trends were observed in the vacuum dried samples. The mechanism of oxalate reduction by heat has not been fully understood. Generally, the rate of oxalate decomposition was higher in the *L. geoffrayi* species than the *L. aromatica* species.

Mean values for percent soluble oxalate content showed that HA drying temperature results in induction impact in reducing the soluble oxalate content in dried *L. aromatica* and *L. geoffrayi* (Figure 1). The percentage of oxalate content in HA dried *L. aromatica* ranged from 32.38 to 42.83%, having a minimum for the sample with drying temperature of 70°C (32.38%) and maximum for the sample with drying temperature at 50°C (42.83%). In the case of HA drying of *L. aromatica*, percent soluble oxalate content decreased when the drying temperature increased from 50 to 70 °C and then increased slightly at 80°C. For the drying of *L. geoffrayi*, percent soluble oxalate content significantly decreased when the drying temperature increased from 50 to 70°C, and then decreased slightly and non-significant at 80°C. In the case of vacuum drying, mean values for percent soluble oxalate content showed that vacuum drying temperature results in non-significant induction impact in reducing the percent soluble oxalate content in dried *L. aromatica* and *L. geoffrayi*. In a sense, higher drying temperature leads to lower components degradation, which was attributed to shorter drying time. In vacuum condition leads to lower components degradation too, which was attributed to low oxygen concentration in the drying chamber. Heat and oxygen transform ascorbic acid or sodium ascorbate to dehydroascorbic acid, which is degraded further to oxalic acid or sodium oxalate (Bauernfeind, 1982)

Table 1 Oxalate contents (mg/g DW) in *L. aromatica*

Sample	*L. aromatica*			
	Moisture content (%) [NS]	**Total oxalate**	**Soluble oxalate**	**Insoluble oxalate**
HA, 50°C	6.95±0.05	36.68±2.86^{abc}	15.65±0.29^{d}	21.03±2.84^{abc}
HA, 60°C	6.90±0.08	36.60±1.06^{abc}	13.30±0.17^{e}	23.30±0.90^{ab}
HA, 70°C	6.87±0.09	35.20±1.38^{c}	11.39±0.04^{f}	23.82±1.35^{a}
HA, 80°C	6.89±0.10	33.05±2.67^{cd}	11.75±0.25^{f}	21.30±2.85^{abc}
VC, 50°C	6.95±0.05	34.16±1.61^{cd}	16.51±0.19^{c}	17.65±1.57^{c}
VC, 60°C	6.92±0.03	36.05±3.44^{bc}	16.93±0.45^{bc}	19.12±3.09^{abc}
VC, 70°C	6.89±0.08	40.92±4.76^{ab}	17.57±0.34^{ab}	23.35±4.89^{ab}
VC, 80°C	6.91±0.05	41.61±3.32^{a}	18.23±0.99^{a}	23.38±2.46^{ab}

Note: Values are expressed as means ± standard deviation (n=3)

[a, b] Values in the same column followed by different letters are significantly different (p<0.05)

NS = not statistically different (p>0.05).

Table 2 Oxalate contents (mg/g DW) in *L. geoffrayi*

Sample	*L. geoffrayi*			
	Moisture content (%) [NS]	**Total oxalate**	**Soluble oxalate**	**Insoluble oxalate**
HA, 50°C	6.92±0.06	29.17±1.19^{b}	21.03±2.84^{abc}	19.94±0.41^{d}
HA, 60°C	6.88±0.07	26.03±0.90^{cd}	23.30±0.90^{ab}	20.13±0.73^{d}
HA, 70°C	6.84±0.08	25.33±0.64^{de}	23.82±1.35^{a}	21.85±0.56^{cd}
HA, 80°C	6.87±0.06	23.03±0.87^{e}	21.30±2.85^{abc}	19.89±0.75^{d}
VC, 50°C	6.90±0.06	28.14±0.91^{bc}	17.65±1.57^{c}	24.08±0.96^{bc}
VC, 60°C	6.89±0.09	30.42±0.73^{b}	19.12±3.09^{abc}	26.01±0.72^{ab}
VC, 70°C	6.83±0.08	32.82±1.22^{a}	23.35±4.89^{ab}	27.78±1.22^{a}
VC, 80°C	6.82±0.11	33.90±0.66^{a}	23.38±2.46^{ab}	28.04±0.77^{a}

Note: Values are expressed as means ± standard deviation (n=3)

[a, b] Values in the same column followed by different letters are significantly different (p<0.05)

NS = not statistically different (p>0.05).

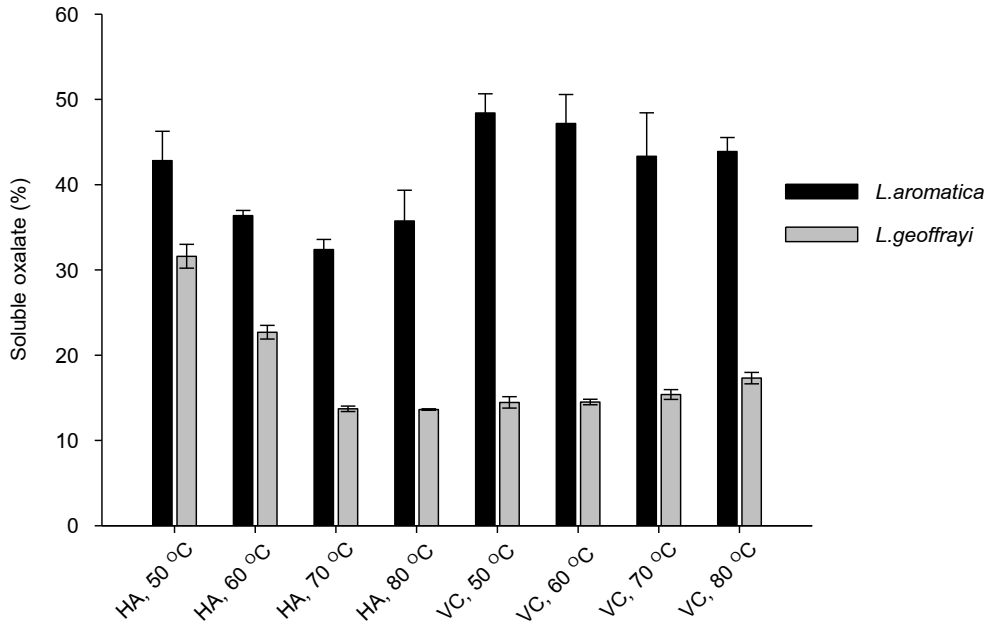

Figure 1 Percentage of soluble oxalate contents in dried *L. aromatica* and *L. geoffrayi*

3.3 Insoluble oxalate content

The values of insoluble oxalate in fresh *L. aromatica* and *L. geoffrayi* were not significantly different (47.74 mg/g dry basis and 45.18 mg/g dry basis respectively) (data not shown). Insoluble oxalate content of *L. aromatica* and *L. geoffrayi* as affected by different drying temperatures are presented in Table 1 and 2. The results reveal that the value of insoluble oxalate in dried *L. aromatica* and *L. geoffrayi* are not significantly different. In the case of HA drying, the insoluble oxalate content in dried *L. aromatica* ranges from 21.03 to 23.82 mg/g DW, having a minimum for the sample with drying temperature at 50°C (21.03 mg/g DW) and maximum for the sample with drying temperature at 70°C (23.82 mg/g DW), *L. geoffrayi* ranged from 19.84 to 21.85 mg/g DW, having a minimum for the sample with drying temperature at 80°C (21.03 mg/g DW) and maximum for the sample with drying temperature at 70°C (23.82 mg/g DW). In the case of vacuum drying, insoluble oxalate content non-significant increased when the drying temperature increased from 50 to 80°C (Table 1 and 2). Oxalates are known to interfere with calcium absorption by forming insoluble salts with calcium. However, a diet containing foods rich in insoluble oxalates is not likely to be harmful. For the results, insoluble oxalates in vegetables converted to soluble oxalates as the sample was dried. Oxalates are known to interfere with calcium absorption by forming insoluble salts with calcium (Holloway *et al*., 1989). When heated, calcium oxalate monohydrate loses its water of crystallization to form anhydrous calcium oxalate. Calcium oxalate then thermally decomposes to calcium carbonate with the loss of carbon monoxide. Finally involves thermal

decomposition of calcium carbonate to calcium oxide with the loss of carbon dioxide (Haines, 2002). The distinction is not permanent because conversion between the forms happens easily (Kumoro, 2012). Some plants contain oxalate oxidase enzymes that breakdown oxalate to carbon dioxide and hydrogen peroxide (Suguira *et al*., 1979). The soluble form can dissolve completely in liquid, meaning that it can pass across the body's barriers quite easily. Kidney stone patients are advised to avoid high oxalate containing food. However, a diet containing food rich in insoluble calcium oxalate is probably not harmful.

An oxalate restriction is defined as a limit of dietary oxalate to no more than 40 to 50 milligrams per day (Massey, 2007). Only <10% of ingested oxalate is absorbed (Borghi *et al*., 2006). This may render dietary oxalate restrictions rather ineffective. Moreover, determining exact oxalate consumption is difficult as there are variations in food levels, the environment in which the food is grown, and the levels also depend on method of analysis used and cooking methods (Grases *et al*., 2006; Massey, 2007). The first step in a dietary oxalate restriction consists of limiting the foods highest in oxalate (Massey, 2007). Overall the oxalate content of *L. aromatica* and *L. geoffrayi* are close to the levels found in some Thai vegetables (Judprasong et al., 2006). Thai cuisine originally used them for their flavoring and aroma properties, and so they are usually consumed in small amounts (about 0.5 grams of dried spice) within larger mixed dishes. Regular consumption of *L. aromatica* and *L. geoffrayi* would not significantly increase the daily intake of oxalates in the diet and therefore would not pose any risk to people who are at risk of forming kidney stones.

4. Conclusions

In summary, our study has demonstrated that various drying methods and temperature influence the contents of soluble and insoluble oxalates of dried *L. aromatica* and *L geoffrayi*. Overall, increased drying temperature decreases the soluble oxalate content of hot-air dried samples, while they were increased with the vacuum dried samples. According to the results from our present study, hot-air drying should be considered as a suitable drying methods for rice paddy herbs with respect to oxalates content. In addition, the soluble and total oxalate contents of various drying temperature of the *L. geoffrayi* specie were significantly lower than the *L. aromatica* species. In terms of health, the soluble oxalate contents of *L. geoffrayi* extracts were greater than those of *L. aromatica* extracts in all samples studied. Clarifying whether cooking these vegetables would further reduce the soluble oxalate content is necessary for further studies.

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