Oxalate contents of Thai rice paddy herbs (*L. aromatica* and *L. geoffrayi*) are affected by drying method and changes after cooking

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Abstract

Food processing, including drying and cooking, generally influences the physical and chemical qualities of food products. The present study assessed the changes in oxalate contents of two rice paddy herbs (phak-kha-yaeng in Thai), *Limnophila aromatica* (phak-kha-yaeng khao) and *Limnophila geoffrayi* (phak-kha-yaeng daeng), as affected by different drying methods, namely hot-air, freeze and vacuum drying. The entire, soluble and insoluble oxalate contents of the cooked samples were also evaluated. Dried samples showed a significant increase in soluble oxalate content compared to fresh samples except for hot-air dried phak-kha-yaeng daeng, while entire and insoluble oxalate contents in dried samples were significantly decreased. Levels of soluble oxalates which were 30% in fresh phak-kha-yaeng khao and 46% in fresh phak-kha-yaeng daeng can be significantly reduced after cooking by boiling. In addition, the cooking of both the hot-air dried phak-kha-yaeng samples also showed greater losses in oxalates than those of other samples studied. Hot-air drying should be considered as a suitable drying method for phak-kha-yaeng with respect to reducing its oxalates. The present study has provided useful information for the drying of vegetables or plant foods to reduce the risk of consuming plant foods which contain high levels of oxalates.

Keywords Freeze drying · Vacuum drying · Vegetables · Soluble oxalate · Cooking

1 Introduction

Oxalate is widely dispersed in plant foods in an easily water-soluble form as potassium, sodium and ammonium oxalates and as insoluble calcium oxalates [1, 2]. Oxalate forms robust chelates with dietary calcium, consequently rendering the complicated molecule 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 recommended to restrict the consumption of oxalate-rich foods, particularly for individuals at risk of kidney stone formation [3]. A diet high in soluble oxalates

is well known to be related to an accelerated risk of developing kidney stones, with the main type being composed in particular of crystals of calcium oxalate [4]. Vegetables are typically consumed as sources of vitamins, minerals, antioxidants and dietary fiber. Some plant ingredients are widely known to contain oxalates [5, 6] which can be recognized as inhibitors of mineral bioavailability.

Several species of *Limnophila*, known as “rice paddy herb” or “phak-kha-yaeng” (in Thai), have been used as a spice and medicinal plant in Southeast Asia. Two species of phak-kha-yaeng are commonly found in Thailand, being phak-kha-yaeng khao (*L. aromatica*) and phak-kha-yaeng daeng (*L. geoffrayi*), which have been used for flavoring and aroma properties. These plants are easily cultivated

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in flooded rice fields and soggy land. The plant is eaten both raw and steamed. It is used in Thai cuisine to add flavor to Thai foods. Especially in northeastern Thailand, phak-kha-yaeng is among the popular ingredients used as a vegetative ingredient in traditional Thai curry. Cultivated phak-kha-yaeng khao plants, have been shown to have negligible toxicity and possess diuretic, muscle relaxant, and antispasmodic activities. They have been used to treat kidney stones, painful cramps, for wound care and ulcers [7]. Phak-kha-yaeng daeng which grows naturally is used as an antidote for poison detoxification and is considered a vegetable in northeastern Thailand. This species is also used as a traditional medicine due to its antipyretic, expectorant, and galactagogue properties [8].

These vegetable plants can be used fresh or dried as a spice. However, the fresh vegetables have a very short post-harvest life of 2–3 days, 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 dried spices. The quality of the dried product strongly depends on the drying conditions. In the process of drying, heat is required to evaporate moisture from the product and a flow of air to carry away the evaporated moisture, making drying a high energy consumption operation [9]. There are different heat resources available for drying and these are well discussed in many articles [10]. Traditionally, the drying process has involved solar drying, however this method is time consuming and weather dependent. Hot-air drying is the most typically employed commercial method for drying vegetables and fruits [11, 12]. Freeze drying is a gentle dehydration technique representing the process of removing the moisture by sublimation of a frozen product. This technique has the ability to slow the product quality deterioration reactions, owing to both its low processing temperature and the virtual absence of oxygen during processing [13]. Previous research has reported that vacuum-dried products are characterized by their better quality retention relative to hot air drying under atmospheric pressure. Vacuum drying has been found to be more effective in drying material with a higher amount of free moisture, such as mango pulp [14], date powder [15], onion slices [16] and yacon slices [17]. However, information about the drying process on oxalate content of vegetable products, especially rice paddy herbs, has not been properly documented. Therefore, the aim of the present study was to assess the influence of different drying methods on oxalate content, including soluble, insoluble and total oxalate contents from two phak-kha-yaeng plants (*L. aromatica* and *L. geoffrayi*) and to investigate the effect of cooking on these substances. The effects of hot-air drying, freeze drying and vacuum drying were investigated for optimizing the most suitable drying methods. We hope to provide

useful information for the drying of vegetables or plant foods to reduce the risk of consuming plant foods containing high oxalate content.

2 Materials and methods

2.1 Sample preparation

Phak-kha-yaeng samples include two species: *Limnophila aromatica* (Lamarck) Merrill (phak-kha-yaeng khao) and *Limnophila geoffrayi* Bonati (phak-kha-yaeng daeng). Phak-kha-yaeng khao samples were bought from a market in Kalasin Province, Thailand, and phak-kha-yaeng daeng samples were obtained from northeastern Thailand, in March 2017. Phak-kha-yaeng samples were cleaned and the edible parts were separated. The raw samples were washed and kept at room temperature to drain. Afterwards, the samples were dried under hot-air, freeze dried and vacuum dried. All analytical results were expressed as a dry matter basis and performed in triplicate.

2.2 Drying processes

Samples were subject to three different drying methods, i.e., hot-air, freeze drying and vacuum drying. For each drying method, 100 g of fresh sample was used (in triplicate). The protocols used in this present study were selected from the optimal conditions of each method, which were preliminarily studied in our lab. In hot-air drying, the sample was dried using the hot-air oven (Thermotec 2000 oven, Conterm, New Zealand) at 70 °C. In freeze drying, the sample was frozen at –20 °C for at least 3 h and then lyophilized using a freeze dryer (Heto, Power Dry, PL300, Freeze Dryer) at –60 °C for 24 h. For vacuum drying, the sample was dried in the vacuum dryer at 70 °C and 0.025 mbar vacuum pressures using a vacuum oven (VD23, Binder, Germany). Drying time was set to achieve dried samples containing 7% moisture content. After drying, these samples were subjected to cool to ambient temperature before extraction.

2.3 Cooking

Ten grams of edible part samples were boiled in deionized water. The amount of water used for cooking was as necessary to cover the amount of each sample studied. The ratios between sample and boiled water were 1:10 and 1:50 (w/v) for fresh and dried samples, respectively. After 10 min, the samples were taken and the cooking water was discarded by allowing the sample to drain and cool to ambient temperature before extraction.

2.4 Oxalate extraction

The total and soluble oxalate contents in test samples were extracted using a modification of the procedure described by Savage, Vanhanen [5]. One gram of each finely ground dried sample was extracted with 50 mL HCl (2 M) in a water bath 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 a 100 mL volumetric flask 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 percentage of soluble oxalate content was calculated by (soluble oxalate/total oxalate) × 100. The insoluble oxalate content was calculated by the difference between the total and the soluble oxalate content. Samples were analyzed in triplicate.

2.5 Oxalate determination

Analysis of total and soluble oxalates was carried out using a 15 cm × 4.6 mm, 5 µm 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, UV-Diode Array detection at 210 nm. Standard curves were prepared using 0.2 M HCl and nanopure water for total and soluble oxalate.

2.6 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 level was 95% ($p < 0.05$).

3 Results and discussion

Oxalates assessment of fresh phak-kha-yaeng and that dried using different drying methods (hot-air, freeze and vacuum drying) was performed for soluble, insoluble and total oxalate content. Moisture content was fixed at 7% dry basis, according to the industrial standards for herbs and spices dried products [18]. The average initial moisture content from the fresh samples of phak-kha-yaeng khao and phak-kha-yaeng daeng were 89.95 and 85.72% wet basis, respectively. The time required to achieve the above levels of moisture content were 3 h for hot-air drying, 24 h for freeze drying and 5 h for vacuum drying.

3.1 Effect of drying method on oxalates

Oxalate contents of phak-kha-yaeng dried by different methods compared to fresh samples are shown in Table 1. Overall, phak-kha-yaeng khao had the higher values of soluble oxalates (8.95–14.57 mg/g DW) compared to phak-kha-yaeng daeng (3.47–5.32 mg/g DW). The drying methods used for drying of the two phak-kha-yaeng types show significantly different soluble oxalates ($p < 0.05$). Dried phak-kha-yaeng showed a significant increase in soluble oxalate content compared to fresh samples, except for hot air dried phak-kha-yaeng daeng, while total and insoluble oxalate contents in dried samples were significantly decreased. The results presented in this work suggest that the changes in total and insoluble oxalates of hot air dried phak-kha-yaeng daeng were larger as compared to freeze and vacuum drying. 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, this 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 [19]. Oxalic acid forms water-soluble salts with Na⁺, K⁺ and 4 ions; it can also form insoluble

Table 1 Oxalate contents (mg/g DW) in fresh and dried phak-kha-yaeng

Samples	Total oxalate		Soluble oxalate		Insoluble oxalate	
	phak-kha-yaeng khao	phak-kha-yaeng daeng	phak-kha-yaeng khao	phak-kha-yaeng daeng	phak-kha-yaeng khao	phak-kha-yaeng daeng
Fresh	56.03 ± 0.99 ^a	48.55 ± 1.44 ^a	8.95 ± 0.69 ^c	4.91 ± 0.09 ^b	47.04 ± 1.41 ^a	43.64 ± 1.35 ^a
Freeze dried	40.46 ± 0.21 ^b	39.03 ± 0.31 ^b	11.35 ± 0.18 ^b	5.32 ± 0.22 ^a	29.11 ± 0.12 ^b	33.72 ± 0.11 ^b
Hot-air dried	35.20 ± 1.38 ^c	29.66 ± 0.23 ^c	11.39 ± 0.04 ^b	3.47 ± 0.12 ^c	23.82 ± 1.35 ^d	26.19 ± 0.29 ^c
Vacuum dried	40.92 ± 1.36 ^b	39.82 ± 0.33 ^b	14.57 ± 0.34 ^a	5.04 ± 0.04 ^b	26.35 ± 2.31 ^c	34.78 ± 0.36 ^b

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$

oxalates by binding with Ca^{2+} , Fe^{2+} and Mg^{2+} rendering these minerals unavailable [5]. 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 [3, 5, 20, 21]. Moreover, the oxalate content of foods can vary depending on the variety, growth, season, soil conditions, time of harvest and many other factors [22, 23]. Percentage of soluble oxalate contents in phak-kha-yaeng before and after cooking were comparing to the total oxalate content of each samples. Mean values for percent soluble oxalate content show that the hot air drying method results in a larger reduction in soluble oxalate content after cooking dried phak-kha-yaeng khao and phak-kha-yaeng daeng compared to the other dried samples studied (Table 2).

Dried phak-kha-yaeng khao and phak-kha-yaeng daeng showed a significant decrease in insoluble oxalate content compared to fresh samples. The different drying methods for phak-kha-yaeng khao showed significant ($p < 0.05$) differences in insoluble oxalate, while vacuum and freeze drying methods of phak-kha-yaeng daeng showed no significance. Oxalates are known to interfere with calcium absorption by forming insoluble salts with calcium. However, diets containing foods rich in insoluble oxalates are not likely to be harmful. The results indicate that insoluble oxalates in vegetables are converted to soluble oxalates as the sample is dried. The degradation of oxalate occurs through oxidation, decarboxylation, and acetylation.

Oxalate oxidase, which is one of the most important oxalate-degrading enzymes, breaks down oxalate to carbon dioxide and hydrogen peroxide. The insoluble oxalates are highly correlated with the insoluble calcium. If this form of calcium is to be utilized by the plant, an enzymatic mechanism or some other process would have to change the insoluble calcium oxalates to other soluble compounds [24]. Oxalates are known to interfere with calcium absorption by forming insoluble salts with calcium [1]. Kidney stone patients are advised to avoid high oxalate containing food. However, diets containing food rich in insoluble calcium oxalate are probably not harmful.

3.2 Effect of cooking on oxalates

Table 3 shows the influence of cooking by boiling on oxalate contents in phak-kha-yaeng khao and phak-kha-yaeng daeng. Overall, cooking by boiling resulted in losses of total, soluble and insoluble oxalate contents compared to uncooked phak-kha-yaeng samples studied. Comparison of oxalate content between before and after cooking showed a greater level of total, soluble and insoluble oxalate contents in both the hot-air dried phak-kha-yaeng samples (Tables 1, 3). In addition, the results show that cooked samples from different drying methods used for the dehydration of phak-kha-yaeng showed significantly different oxalate contents ($p < 0.05$). Percentage losses for soluble oxalates of fresh, freeze dried, hot air dried and

Table 2 Percentage of soluble oxalate contents in phak-kha-yaeng before and after cooking by boiling

Samples	Before cooking		After cooking	
	phak-kha-yaeng khao	phak-kha-yaeng daeng	phak-kha-yaeng khao	phak-kha-yaeng daeng
Fresh	15.97 ± 0.96 ^d	10.11 ± 0.14 ^d	16.39 ± 0.64 ^c	7.35 ± 0.32 ^c
Freeze dried	28.05 ± 0.34 ^c	13.62 ± 0.46 ^a	30.64 ± 0.76 ^a	13.24 ± 0.43 ^a
Hot-air dried	32.38 ± 1.19 ^b	11.70 ± 0.43 ^c	28.25 ± 0.36 ^b	7.47 ± 0.11 ^c
Vacuum dried	35.64 ± 1.82 ^a	12.66 ± 0.18 ^b	27.84 ± 1.29 ^b	10.71 ± 1.13 ^b

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$

Table 3 Oxalate contents (mg/g DW) in fresh and dried phak-kha-yaeng after cooking by boiling

Samples	Total oxalate		Soluble oxalate		Insoluble oxalate	
	phak-kha-yaeng khao	phak-kha-yaeng daeng	phak-kha-yaeng khao	phak-kha-yaeng daeng	phak-kha-yaeng khao	phak-kha-yaeng daeng
Fresh	37.94 ± 1.22 ^a	35.99 ± 0.62 ^a	6.22 ± 0.32 ^b	2.64 ± 0.07 ^b	31.72 ± 1.03 ^a	33.35 ± 0.69 ^a
Freeze dried	25.14 ± 0.04 ^c	24.82 ± 0.04 ^c	7.70 ± 0.20 ^a	1.05 ± 0.11 ^c	17.10 ± 0.76 ^c	21.54 ± 0.11 ^b
Hot-air dried	16.86 ± 0.22 ^d	13.75 ± 0.21 ^d	4.76 ± 0.01 ^c	0.28 ± 0.01 ^d	12.10 ± 0.22 ^d	12.72 ± 0.21 ^c
Vacuum dried	27.42 ± 1.35 ^b	27.10 ± 0.21 ^b	7.62 ± 0.02 ^a	4.07 ± 0.01 ^a	19.80 ± 1.34 ^b	24.19 ± 0.37 ^b

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$

Fig. 1 Percentage loss of soluble oxalate content for fresh and dried phak-kha-yaeng samples after cooking by boiling, compared to uncooked samples

Fig. 2 Percentage loss of total oxalate content for fresh and dried phak-kha-yaeng samples after cooking by boiling, compared to uncooked samples

vacuum dried phak-kha-yaeng after cooking by boiling compared to samples before being cooked are shown in Fig. 1. There were losses in soluble oxalate value following fresh, freeze dried, vacuum dried and hot air dried phak-kha-yaeng khao (fresh: 30.45, freeze dried: 32.14, vacuum dried: 47.66 and hot-air dried: 58.17%). In phak-kha-yaeng daeng, the percentage loss of soluble oxalates increased in the following order: freeze dried < vacuum dried < fresh < hot-air dried samples (freeze dried: 38.15, vacuum dried: 42.43, fresh: 46.07 and hot-air dried: 70.37%). The results for percent reduction of total oxalates due to cooking of phak-kha-yaeng khao and phak-kha-yaeng daeng show that hot air dried samples had higher losses of total oxalate (52.03 and 53.63%) compared to freeze dried (37.87 and 36.40%), vacuum dried (32.98 and 31.94%) and fresh samples (32.27 and 25.83%) respectively (Fig. 2). High temperatures are known to cause the calcium oxalate containing cells to collapse, leading to the

breakdown of the oxalate structure [25]. When Mosha et al. [26] prolonged thermal processing; they observed increased losses in oxalates, as was also found in the present study. It can be assumed that this was due to the low solubility of these compounds and their resistance to the effect of temperature [27]. The chemical reactions and changes in the permeability of cell membranes taking place during processing may have increased the leaching of oxalates. Losses of oxalates in all samples studied are likely due to their leaching loss in the cooking water. However, different drying methods or pretreatment used in phak-kha-yaeng showed different loss value of oxalates; this may have been due to the conversion of soluble oxalates during the drying of the samples to insoluble oxalate initiated by heating conditions.

An oxalate restriction is defined as a limit of dietary oxalate to no more than 40–50 mg/day [21]. Only < 10% of ingested oxalate is absorbed [28]. This may render dietary oxalate restrictions rather ineffective. Moreover, determining exact oxalate consumption is difficult as there are variations in food levels, the environment wherein the food is grown, and the levels also depend on evaluation methods used and cooking methods [21, 29]. The first step in a dietary oxalate restriction consists of limiting the foods highest in oxalate [21]. Overall the oxalate contents of the two phak-kha-yaeng are close to the levels found in some Thai vegetables [30]. Regular consumption of phak-kha-yaeng khao and phak-kha-yaeng daeng 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

The present study has demonstrated that different drying methods resulted in different oxalate contents. Overall, the phak-kha-yaeng samples hot-air dried had lower content of total and insoluble oxalates, compared with freeze dried and vacuum dried samples, as well as with fresh samples. The soluble oxalate contents of fresh samples were greater than those of dried samples in all samples studied, except for the hot air dried phak-kha-yaeng daeng. Levels of soluble oxalates of more than 30% can be significantly reduced after cooking by boiling. Both the cooked hot air dried phak-kha-yaeng samples also showed greater losses in oxalates than those of the samples studied. According to the results from our present study, hot air drying should be considered as a suitable drying method for phak-kha-yaeng with respect to reducing its oxalates. The results obtained demonstrate that greater care should be taken in the preparation as well as drying and cooking of vegetables containing oxalates, and these vegetables should only

be consumed in small amounts, especially for individuals at risk of kidney stone formation.

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Compliance with ethical standards

Conflict of interest This manuscript have no conflict of interest.

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