#### LWT - Food Science and Technology 50 (2013) 259-263



Contents lists available at SciVerse ScienceDirect

#### LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

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## Effect of simple processing methods on oxalate content of taro petioles and leaves grown in central Viet Nam

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#### ARTICLE INFO

Article history: Received 8 February 2012 Received in revised form 20 April 2012 Accepted 22 May 2012

Keywords: Taro Petioles Leaves Total Soluble Insoluble oxalates Wilting Soaking Cooking

#### 1. Introduction

Taro (*Colocasia esculenta*) is a major tropical crop which originates from the tropical region between India and Indonesia (Matthews, 2004). It has been grown in the South Pacific for hundreds of years (FAO, 1992). In central Viet Nam, taro is grown as an intercropping plant with sweet potato, maize, cassava, legumes, sugar cane or vegetables. It can be cultivated in wet land, sandy soil, in paddy fields or in gardens (Toan & Preston, 2007). Taro is used for human consumption and animal feed. In Thua Thien Hue province, eight widely grown varieties are given local names, five of these cultivars, Ao Trang, Ngot, Chia Voi, Tim and Nuoc, are grown extensively as forage feeds for pigs (Hang & Preston, 2009, 2010; Toan & Preston, 2007) the other cultivars are grown for their tubers and as vegetables for humans.

Oxalic acid is an organic acid found in many higher plants, including a large variety of commonly consumed food plants. It occurs as the free acid, soluble salts of potassium and sodium, and insoluble salts of calcium, magnesium and iron (Noonan & Savage, 1999). High oxalate concentrations in the leaves of plants

#### ABSTRACT

This study investigated the total, soluble and insoluble oxalate contents of the petioles and the leaves from two different cultivars, Mon Cham (purple stem) and Chia Voi (light green stem), of taro (*Alocasia odora*) grown in central Viet Nam. These cultivars were processed by wilting for 18 h, resulting in an overall 5.9% reduction of soluble oxalates and washing in cold water for 5 min, resulting in a 26.2% reduction in soluble oxalate content. Soaking the petioles and the petioles and leaves for 10 h in water kept at 36-38 °C resulted in a mean 69.5% reduction in the soluble oxalate content of the raw tissues. Boiling for 60 min was the most effective way to reduce the soluble oxalate levels in the cooked tissue. A mean 84.2% reduction in soluble oxalate in the petioles and the petioles and leaves was achieved after boiling for 60 min while mean reductions of 62.1% were achieved when both materials were boiled for only 10 min.

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consumed regularly are of concern because of the harmful health effects associated with the intake of high amounts of oxalates. A diet high in soluble oxalates is widely known to cause an excessive urinary excretion of oxalate (hyperoxaluria) with an increased risk of developing kidney stones. Therefore, people predisposed to forming kidney stones are recommended to minimise their intake of foods high in oxalates (Massey, 2003). Earlier studies have shown that taro leaves contain high levels of soluble and insoluble oxalates (Bradbury & Nixon, 1998; Mårtensson & Savage, 2008; Oscarsson & Savage, 2007; Savage & Dubois, 2006).

Most taro cultivars taste acrid and can cause swelling of the lips, mouth and throat if eaten raw (Bradbury & Nixon, 1998). The acridity is caused by needle-like calcium oxalate crystals known as raphides, which can penetrate soft skin (Bradbury & Nixon, 1998). Both the tubers and the leaves can give this reaction (FAO, 1992) so it is clear that high levels of oxalates in taro leaves and tubers are a significant anti-nutritive factor (Oscarsson & Savage, 2007). The effect of these oxalates in the tissue can be reduced by cooking (Bradbury & Nixon, 1998). Boiling can reduce the oxalate content of a food if the cooking water is discarded; washing and soaking the leaves can also reduce the soluble oxalate content (Noonan & Savage, 1999).

While taro leaves are an important part of Pacific Island culture (Oscarsson & Savage, 2007) the leaves are more often used for

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<sup>0023-6438/\$ –</sup> see front matter  $\odot$  2012 Elsevier Ltd. All rights reserved. doi:10.1016/j.lwt.2012.05.015

feeding animals in Viet Nam (Hang, Binh, Preston, & Savage, 2011). However, taro stems (petioles) are also sold fresh in 500 g bundles in local markets and in some supermarkets in Viet Nam. The skin is removed from the petioles, which are then chopped into small pieces, boiled in water for 15 min with salt, fish, pineapple and tomatoes to make the traditional dish "canh chua bac ha". Taro petioles are also often chopped into small pieces and cooked with pork ribs or chopped and cooked with rice and mussels to make "com hen" a popular dish in Hue city, Viet Nam. Spinach (*Spinacia oleracea*), a comparable high oxalate-containing leafy vegetable, is also used to make a well-known dish Rau Muống in Viet Nam. This dish is cooked in a wok with a number of different local vegetables.

Taro leaves could be used as a human food as they have been shown to be effective as partial or complete substitutes for conventional diets given to pigs and ducks (Giang, Preston, & Ogle, 2010; Nouphone & Preston, 2011; Rodríguez, Lopez, Preston, & Peters, 2006; Tiep, Luc, Tuyen, Hung & Tu, 2006; Ty, Borin, & Preston, 2009). Hang et al. (2011) have shown that initial processing of taro leaves can considerably reduce the soluble oxalate content of processed leaves. The levels of oxalates in the cooked and processed leaves appear to have similar levels to cooked taro leaves grown and processed in New Zealand (Oscarsson & Savage, 2007) or the levels found in cooked silver beet leaves, a vegetable dish consumed in New Zealand (Simpson, Savage, Sherlock, & Vanhanen, 2009).

Since taro grows readily in many different environments in Viet Nam it might be possible to encourage its use as a human food as the leaves contain a wide range of useful nutrients. However, the high oxalate content may increase the risk of kidney stone formation in susceptible individuals and decrease calcium availability through soluble oxalate binding to dietary calcium in the digestive tract. Since insoluble oxalate is unlikely to be absorbed from the intestinal tract (Simpson et al., 2009), these studies were commenced to investigate effective ways to reduce the soluble oxalate content of taro leaves and petioles by processing and cooking so that they can be recommended as a vegetable to supplement the diet.

#### 2. Materials and methods

Two varieties of taro, Mon Cham (purple stem) and Chia Voi (light green stem) (*Alocasia odora* C. Koch) were grown in sandy soil and were collected at the end of May 2010 from three different farms in the Thua Thien district of Hue province, Viet Nam.

Three kg of each variety was sampled at a mature stage of leaf growth from each location. Samples were collected from Thuy An village for the washing and wilting experiment, Thuy Duong village for the soaking experiment and Quang Tho village for the cooking experiment. The leaf samples were sealed in plastic bags and stored at room temperature until analysis commenced the following day. The Mon Cham sample consisted of petioles alone while the Chia Voi sample consisted of 70–75% petioles and 25–30% leaves.

#### 2.1. Processing treatments

#### 2.1.1. Wilting

Leaves and petioles were chopped into 10–20 mm portions and then spread out on a plastic sheet in the shade (under a roof) and allowed to wilt at 37-38 °C for 18 h. Samples were then taken for dry matter analysis prior to drying at 65 °C for 18 h.

#### 2.1.2. Washing

Representative portions of leaves and petioles were chopped into 10–20 mm pieces. One kg of chopped pieces were placed in 5 L cold tap water and washed for 5 min. The chopped pieces were then allowed to drain at room temperature for 30 min. Samples were then taken for dry matter analysis prior to drying at 65  $^\circ C$  for 18 h.

#### 2.1.3. Soaking

Three kg of the 10–20 mm portions were placed in 10 L of tap water at 36-38 °C. Representative samples of the soaked material were taken after 1, 3, 5, 7 and 10 h and then dried at 65 °C for 18 h.

#### 2.1.4. Cooking

Two kg of the 10–20 mm portions were boiled in 4 L tap water. After 10, 30 and 60 min, representative samples were taken and the cooking water was discarded by allowing the sample to drain and cool for 10 min. This sample was then dried at 65 °C 18 h.

#### 2.2. Sample preparation

Each dried sample was ground to a fine powder using a Sunbeam multi grinder (Model no. EMO 400 Sunbeam Corporation Limited, NSW, Australia) and the residual moisture was determined in triplicate (AOAC, 2002), by drying to a constant weight in an oven at105 °C for 24 h.

#### 2.3. Oxalate determination

The total and soluble oxalate contents of 0.5 g of each finely ground sample were determined in triplicate using the method outlined by Savage, Vanhanen, Mason, and Ross (2000). Three separate 0.5 g samples of dried ground sample were placed in a 100 ml flask, 40 ml Nanopure water (Barnstead II, Thermo Fisher Scientific Australia Pty Ltd., Scoresby Victoria, Australia) added and incubated in a water bath at 80 °C for 15 min to extract soluble oxalates. Total oxalates were extracted using 40 ml 0.2 Mol/L HCL at 80  $^{\circ}$ 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 extracts were centrifuged at 2889 rcf for 15 min. The supernatant was filtered through a 0.45 mm cellulose nitrate filter. The chromatographic separation was carried out using a 300  $\times$  7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, Torrance, CA, USA) attached to a cation H+ guard column (BioRad, Richmond, California, USA). The analytical column was held at 25 °C. The equipment consisted of an auto sampler (Hitachi AS-2000, Hitachi Ltd., Kyoto, Japan), a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, California, USA), a Waters, U6K injector (Waters Inc., Marlborough, Massachusetts, USA), a UV/VIS detector Spectra-Physics SP8450 (Spectra-Physics, San Jose, California, USA) set on 210 nm. Data capture and processing were carried out using a peak simple chromatography data system (SSI Scientific Systems Inc, State College, PA, USA). The mobile phase used was an aqueous solution of 25 mM sulphuric acid. Samples (20 ml) were injected onto the column and eluted at a flow rate of 0.6 ml/min. Insoluble oxalate content (calcium oxalate) was calculated by difference (Holloway, Argall, Jealous, Lee, & Bradbury, 1989). The final oxalate values were converted to mg/100 g fresh weight (FW) of the original material, taking into account the moisture content of each sample.

#### 2.4. Statistical analysis

The results are presented as mean values  $\pm$  standard error. Statistical analysis of the total, soluble and insoluble oxalate content of each treatment method was performed using Minitab version 15.1 (Minitab Ltd., Brandon Court, Progress Way, Coventry, UK) using one-way analysis of variance.

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